NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

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RTO MEETING PROCEEDINGS **MP-HFM-108**

NATO Medical Surveillance and Response, Research and Technology Opportunities and Options

(La surveillance médicale et les réponses au sein de l'OTAN: les possibilités et les options pour la recherche et la technologie)

Papers prepared for the RTO Human Factors and Medicine Panel (HFM) Symposium which was held in Budapest, Hungary, 19-21 April 2004.

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The Research and Technology Organisation (RTO) of NATO

RTO is the single focus in NATO for Defence Research and Technology activities. Its mission is to conduct and promote co-operative research and information exchange. The objective is to support the development and effective use of national defence research and technology and to meet the military needs of the Alliance, to maintain a technological lead, and to provide advice to NATO and national decision makers. The RTO performs its mission with the support of an extensive network of national experts. It also ensures effective co-ordination with other NATO bodies involved in R&T activities.

RTO reports both to the Military Committee of NATO and to the Conference of National Armament Directors. It comprises a Research and Technology Board (RTB) as the highest level of national representation and the Research and Technology Agency (RTA), a dedicated staff with its headquarters in Neuilly, near Paris, France. In order to facilitate contacts with the military users and other NATO activities, a small part of the RTA staff is located in NATO Headquarters in Brussels. The Brussels staff also co-ordinates RTO's co-operation with nations in Middle and Eastern Europe, to which RTO attaches particular importance especially as working together in the field of research is one of the more promising areas of co-operation.

The total spectrum of R&T activities is covered by the following 7 bodies:

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS Studies, Analysis and Simulation Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These bodies are made up of national representatives as well as generally recognised 'world class' scientists. They also provide a communication link to military users and other NATO bodies. RTO's scientific and technological work is carried out by Technical Teams, created for specific activities and with a specific duration. Such Technical Teams can organise workshops, symposia, field trials, lecture series and training courses. An important function of these Technical Teams is to ensure the continuity of the expert networks.

RTO builds upon earlier co-operation in defence research and technology as set-up under the Advisory Group for Aerospace Research and Development (AGARD) and the Defence Research Group (DRG). AGARD and the DRG share common roots in that they were both established at the initiative of Dr Theodore von Kármán, a leading aerospace scientist, who early on recognised the importance of scientific support for the Allied Armed Forces. RTO is capitalising on these common roots in order to provide the Alliance and the NATO nations with a strong scientific and technological basis that will guarantee a solid base for the future.

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NATO Medical Surveillance and Response, Research and Technology Opportunities and Options

(RTO-MP-HFM-108)

Executive Summary

Medical Surveillance can comprise all medical information from cradle to grave. NATO Medical surveillance means medical and personnel information systems that are designed, integrated, and utilized with military medical surveillance to protect the physical and mental health of military personnel throughout their military service. This can start with a medical history pre service, and will continue with all data after entering the military, and after separation as a veteran. This data will allow well founded epidemiological research. Depending on the speed of data acquisition and transmission the information may be used for diagnosing unusual events such as an outbreak of an influenza epidemic or a terrorist attack with WMD.

Within the last years, new applications of information technology have expanded our capabilities for surveillance, and these technologies are now starting to move out of hospitals and other fixed facilities into forward deployed settings. Surveillance technology must be developed towards an integrated system of systems that can comprehensively address future needs to identify acute and chronic exposures of military personnel to health threats over the course of their entire military service. Many of the necessary component technologies are available now or will be soon - the key will be to integrate them.

The symposium presented several examples of medical surveillance in a few NATO and PfP nations (US, CA, NL, GR, BU and GEO). Other countries decided not to present at this meeting.

Three areas of surveillance were presented:

- Surveillance technology and philosophies (US, CA, GR, NL)
- Specified surveillance for mental effects of service or deployment (NL, CA, GEO, BU)
- New technologies for rapid diagnosis of infections (US)

The US provided the largest part (20/32 plus two key notes) of the presentations, however they demonstrated also the difficulties in bringing all the systems and information together into a system of systems that is capable of giving all the information to those who need it be he/she medical officer, decision maker or commander in the field.

La surveillance médicale et les réponses au sein de l'OTAN: les possibilités et les options pour la recherche et la technologie

(RTO-MP-HFM-108)

Synthèse

La surveillance médicale peut comprendre l'ensemble des informations médicales de la vie d'un patient. Pour l'OTAN, le terme « surveillance médicale » signifie des systèmes d'informations médicales sur le personnel qui sont conçus, intégrés et utilisés avec la surveillance médicale militaire pour protéger la santé physique et mentale du personnel militaire tout au long de son service militaire. Le processus peut débuter par les antécédents médicaux avant l'entrée en service et se poursuivre jusqu'après la cessation de fonctions pour comprendre les anciens combattants. Les données ainsi accumulées permettent de faire de la recherche épidémiologique sur de bonnes bases. En fonction de la rapidité de l'acquisition et de la transmission des données, les informations peuvent être exploitées pour le diagnostic d'événements inhabituels, tels que la survenue d'une épidémie de grippe, ou un attentat terroriste avec ADM.

Ces dernières années, de nouvelles applications des technologies de l'information ont permis de multiplier les capacités de surveillance qui se trouvaient auparavant en milieu hospitalier et qui sont de plus en plus utilisées par les forces déployées à l'avant. Il est impératif de faire évoluer les technologies de surveillance vers un système de systèmes intégré, en mesure de répondre aux futurs besoins d'identification de cas aigus et chroniques d'exposition du personnel militaire à des menaces pour la santé pendant toute leur carrière. Bon nombre des technologies des composants sont disponibles déjà, ou le seront à court terme – l'élément clé sera de les intégrer.

Le symposium a présenté un certain nombre d'exemples de surveillance médicale dans quelques pays de l'OTAN et du PfP (US, CA, NL, GR, BU et GEO). D'autres pays avaient décidé de ne pas présenter des communications.

Trois domaines de surveillance étaient présentés :

- les technologies et les philosophies de surveillance (US, CA, GR, NL)
- la surveillance spécifique aux effets mentaux du service et du déploiement (NL, CA, GEO, BU)
- les nouvelles technologies pour le diagnostic rapide des maladies infectieuses (US)

Les Etats-Unis ont présenté la majeure partie des communications (20/32 et deux discours d'ouverture). Les conférenciers US ont cependant souligné les difficultés rencontrées pour incorporer toutes les informations et tous les systèmes dans un système de systèmes en mesure de transmettre l'ensemble de ces informations aux demandeurs, qu'ils soient médecins militaires, décideurs ou chefs militaires sur le champ de bataille.

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† Paper not available at the time of publishing.

SESSION II – SURVEILLANCE INFORMATION: TOOLS & TECHNIQUES

SESSION III – LABORATORY TECHNIQUES & TECHNOLOGY FOR SURVEILLANCE

† Paper not available at the time of publishing.

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Technical Evaluation Report

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The following evaluation report is structured in two parts.

Part 1 Evaluation of the papers presented

Part 2 Technical remarks that should help to improve future meetings.

INTRODUCTION

RTO translates technology into military capabilities. Symposia bring together scientists, decision and policy makers. Thus the organizers welcomed observers from the COMEDS WG on Preventive Medicine. One other aim of RTO symposia is to induce networks of scientists in the various armies of NATO Member Nations. This aim has been achieved during this symposium and other contacts have been founded.

The "Operational Medicine" area of the RTO Human Factors and Medicine Panel focuses on all health problems pre-, during and post-deployment in a large group of different Technical Teams. In most cases, their activities are focused on specific health problems. This symposium, however - initiated by the problems of asymmetrical warfare and recent technical developments - attempted to integrate the different aspects of monitoring the soldiers' health status pre-, during and post-deployment. Especially because of the problems that are encountered during worldwide deployments there was a need for a comprehensive view of all aspects of health protection. These topics must not be seen as individual health problems only, but as those of the population (or society).

The organizers divided the large spectrum of presentations into four sessions:

- Epidemiology Techniques for Surveilling Specific Health Conditions
- Surveillance Information: Tools and Techniques
- Laboratory Techniques and Technology for Surveillance
- Epidemiology techniques for Military Forces Surveillance.

Part 1 Evaluation of the Presentations

A major lesson learned from the Gulf War was the need for a comprehensive deployment health surveillance system. Suspected adverse affects from vaccines and other preventive health measures, and unconfirmed exposures to toxic substances have resulted in long term challenges for military leaders and health care professionals. This is one of the major driving forces for the close surveillance of soldiers during deployment.

The purpose of this Symposium was to bring together Public Health, Occupational Health and Military Health professionals to discuss the strategies and technologies available to existing and emerging threats.

The specific objectives would be to share expertise and approaches so that the protection of the health of deployed NATO forces might be optimized.

One of the shortcomings, however, of the – otherwise - excellent symposium was the absence of speakers from major European countries including GE, UK, FR, PL, SP, PO, CZ, HU, IT. However, the presence of the delegates of COMEDS WG on Preventive Medicine made an information transfer into their countries possible.

The symposium was to address technology and policy for medical surveillance systems to support the protection of the health of NATO forces across the continuum of operations (e.g., garrison operations through war and relief operations). In addition to considering support for NATO warfare operations, the symposium addressed the technology and policy necessary for sustainment of adequate public health systems and population health in future NATO areas of operation. Finally, the symposium was to address the state-of-art and importance of providing medical aid within the following hours (or minutes) of the occurrence of a catastrophe - this response can make the difference between a successful relief operation and a collection of a large number of casualties.

The first key note on United States Force Health Protection and Deployment Health Policy and Programs: Policy Support through Automation Systems by Mrs. E. EMBREY, Deputy Assistant Secretary of Defence, Force Health Protection and Readiness (US) gave an overview on the US programs. She described a three pillars approach to the most valuable topic the US has the health of its servicemen and women.

The **first pillar**, ensuring a healthy and fit force, focuses on programs that set standards and promote health and fitness force-wide was addressed by the epidemiological papers of the first day trying to approach standards for a mentally healthy service man.

The **second pillar** of force health protection focuses on programs designed to prevent injury and to protect the force against health hazards. The Software systems to fulfill this aim are being developed and in part even fielded. Thus soon the US will have a huge warehouse of data which need further processing. If however the ergonomic needs of the user who has to enter the data are neglected the data may be not trustworthy. Thus there seems to be a need for considering this problem too.

The **third pillar** of force health protection focuses on DoD's programs which provide world class medical and rehabilitative care anywhere in the world will be addressed during the next RTO Symposium in St. Petersburg this summer.

Throughout history military forces have suffered more casualties from endemic disease and accidents than from military action (DNBI). This trend continues in the era of peacekeeping operations and operations other than war (OOTW) that now occupy the alliance. Reducing these diseases or at least giving early warnings and proficient medical support is paramount for the military who rely on manpower und human well being.

The threat may be presented by infections caused by various emerging respiratory pathogens or known biological warfare. Both groups of agents often present themselves initially as influenza-like illnesses. Thus there is interest in a near-real-time system that can monitor affected soldiers and relatively rapidly identify both covert attacks involving biological agents and emerging respiratory pathogens. The advantage of rapid diagnostics under field conditions was addressed in five (rather) sophisticated papers which at the moment stem from research laboratories but soon may be issued for field use.

Gathering the different presentations under single headings one could state that ten papers reported on US efforts for surveillance of the soldier with the aims of giving the commanders the needed information on

actual well being of their soldiers as well as gathering the information in some centralized data banks where all information comes together "from cradle to grave" which make epidemiologic research more effective in the future.

The epidemiological papers with regard to mental health screening deal with the problem that people suffering from mental diseases are stigmatized. Consequently even interrogation may cause stigmatization. If however, a very large group is screened, it is difficult to draw a border line between those who need further observation or even treatment and those who are just a modification in the broad spectrum from normal to ill. It may be overt as in PTSD or more or less hidden in depression, substance abuse, job loss, unemployment, divorce, and spouse abuse. The causes of such deviations from "normality" may on one hand be the unusual situation(s) during deployment with its different physical and social environment or the inability of the individual to cope with these problems the causes of which may be far back in his/her pre-military life. The detailed research done by Canadian, US-American, Bulgarian and Dutch researchers is impressive and demonstrates the borders of medical-social-psychological research. Are those with poor social nets attracted by the military or does the military weaken social resources? Questions very difficult to answer but very important in order to have men and women in the forces that are able to cope with the problems of unsymmetrical war fare.

Apparently, most of the screening tools do not have sufficient sensitivity, specificity and predictive value to base a treatment on it. Long term observations of large cohorts after a standardized screening is needed before we can reach evidence based levels. The symposium offered the opportunity that these scientists met and it may start a common evaluation of the existing data. Additionally, it is possible that the limits and standards for the normal population may not apply to the military.

It is very good to see that small parts of the large mosaic of protecting our troops come from epidemiological studies that per se at first glance appear to have little to do with the war fighter such as papers by ZHORZHOLIANI and by KARSELADZE both from Georgia. Apparently, they have the very good opportunity to study adolescents early in their lives and possibly may follow them through a military career.

Within the area of data collection and processing various systems exist.

GEIS (Global Emerging Infectious Surveillance) as explained by Kenneth COX is aimed at early recognition of epidemics and possible terrorist attacks. It will become a useful tool as soon as data from all over the world are collected and processed.

ESSENCE (Electronic Surveillance System for the Early Notification of Community-based Epidemics) is such a system that, however at the moment is limited to DoD out-patient data. A modification to better differentiate ICD-9 diagnostics on one hand and including data from deployment into it will lead to a much stronger tool. Its value is clearly demonstraqted in a second paper on that topic by Victor MACINTOSH especially when correlated to the CDC data.

TMIP (Theater Medical Information Program) appears to be another alternative and has the advantage that is has been fielded.

JMeWS (Joint Medical Workstation) is part of TMIP. It is Web based and allows not only medical authorities to evaluate the gathered information but also the commanders, thus bridging a gap between the medical and the military part of the forces. Thus it will be a very useful tool for epidemiological studies depending on the data fed into it. It may be thought of including mental health screening data in the system too, so that later this information may be connected to other ones. This will be ideal for researchers but problems with the privacy of such information and their possible misuse may be seen.

MEDBASE presented more like a sales show than scientifically is a system that is aimed at collecting all data of all soldiers without paper and pencil tools. It has been developed for the army, where commanders may control the health status of their soldiers and to avoid all the pitfalls of earlier systems. The link to existing systems and their incorporation or at least use could not be clearly demonstrated. But a bridge may be constructed via the CHCS II.

Another system that has been developed is called MDSS (Medical Data Surveillance System). It showed its immediate use by diagnosing dermatological problems in one unit. After the personnel of the Joint Task Force Headquarters were able to locate the unit, the problem was solved immediately. In this very simple example I dare say that a caring medical officer or nurse, who uses their time reflecting about the data they get instead of typing them into a keyboard might have had the same result. But it is good to know that data processing systems are able to compensate for human failures.

Another paper on that topic (# 12, REIFMAN et al.) was an overview of what should be considered and thought of by critically reviewing the literature. This valuable paper should initiate many new thoughts by the developers of the other systems.

The paper of SCHMORROW et al gave a sight into the future where every soldier has its own data acquisition capability. What will be needed than is an electronic headquarter that filters all incoming data. Additionally the military use may be different. If such an individual data logger and transmitter would reduce the time span between being wounded and receiving first medical treatment it will save many lives. Thus the system must be followed on but there has to be a very effective way of reducing the mass data flow and minimizing the battery weight (lighten the soldier!).

In contrast to the presented surveillance systems for present or future use the EPINATO system was evaluated several years after its development. The results did not show beneficial effects of the system, which is not used by many forces.

A completely different approach of multinational cooperation was presented DIAMANTOPOULOS et al. This multi-nation approach using the principles of telemedicine connecting hospitals in different countries may serve as a model state-of the art medical surveillance system, which owns the potential to be utilized in several ways (e.g. C3, medical planning, casualty management) both in mobile units, and in case of civilian events, such as a major catastrophe with mass destruction.

All these excellent surveillance systems, however, would be less useful if the reaction capabilities would not have been developed in parallel. Thus two originally quite different science branches, information technology and microbiological diagnostic and genetics work hand in hand. It was therefore consequent by the organizers of the symposium to devote a session to the modern detection and diagnostic systems for diseases and possible terrorist attacks with biological MWD.

One major topic that was addressed too little is the user friendliness of the interfaces. All the surveillance systems are only as good as the data are reliable and correct. Since there is little time for training all interface users they have to be constructed according well established rule of software ergonomics and they should be self guiding using the symbols and hot keys that are familiar to them from the home computers.

The advantage of close supervision of the health status of the military allows even now a days the start of thorough epidemiological research. The side effect of this capability can be an early warning - even of the civilian population - of an outbreak of influenza or influenza like diseases.

Health care can be seen as a chain of interventions. They range from indoctrination and training for a healthy life style to hygiene and prevention, to care of the wounded or traumatized, to the rehabilitation of veterans. The specific health interventions were not the part of the symposium, but the systems that can monitor health risks, exposures, or health changes.

One new idea was introduced into a HFM symposium and that is, that the discussion should be included into the proceedings. It has been documented in AGARDOGRAPHs that during the discussion very valuable information is added to the presentation or to the presenter. We are proud that we were able to reach this goal. Thus these proceedings will have an additional wealth of information.

Part 2: Technical Remarks:

One could easily say this Symposium was an US-American event with some international participation. 2 Key Note speakers and 20 presenters came from US which is 65%, 4 presentations were from The Netherlands (12%), 3 presentations came from Canada (9%), 2 from Georgia (6%) and one each (3%) from Greece, and Bulgaria.

Thus it appears to be advisable to ask future program committees to search for papers from more countries and to directly address the national voting members for presentations from their countries. This appears to be the only means to accomplish one of the goals of RTO: “The mission of RTO is to conduct and promote co-operative research and information exchange.”

The fact, however, that this was almost an American event with some international contributions led some native English speakers to forget that RTO is an international auditorium where many participants may be less fluent in the English language. Some of them spoke rather fast; many of them used abbreviations, which may be familiar to US military but not to all NATO countries. Thus they probably did not get all the information they presented to all of the audience, which is a pity.

Some studies on human pathology suffered, I’m happy to admit, from too small numbers. Therefore difficulties arose whether social problems are the consequence of difficulties in coping with traumatic events during deployment or whether the difficulty in coping with extraordinary events pre-existed and was brought into the military life by the soldier himself: Does the military attract people with coping problems or poor social resources? The Dutch answer was: yes!

In this area much research effort has to be accomplished before we can reach evidence based results. Here an international cooperation as has been put forward by this symposium is urgently needed. Thus the symposium has fulfilled its purpose: information has been exchanged and networks may be started. The information exchange has also been documented by the vivid discussions, which are very valuable for the authors as well since they may learn where future research should be directed to.

Another point that has to be mentioned is that some of the slides shown did not follow the rules of ergonomic presentations with respect to letter size, description of abscissas and ordinates and using unexplained abbreviations. If the purpose of a slide is to illustrate und underline the spoken word, a number of presenters forgot about this. If the listener is distracted by poor slides he is less capable to follow the spoken word and the message may get lost, which again is a pity.

RECOMMENDATIONS

- The publication of the presentations should be sent to a wide audience working in the scientific, operational and management field of the NATO military medical services. This can contribute to the lack of interaction due to the cancellation of the symposium.
- Further action should be taken to integrate work on this topic that is done in various countries. Especially the NATO/COMEDS working groups and NATO/NAS should be informed, to make it possible to change NATO procedures.
- Use any effort possible to make all system as the user friendly as possible If the soldier who enters the data does not understand why he/she should enter what and where the input may be wrong and than the whole data will be wrong. So improving surveillance and data collections systems may result in poor data if the are typed incorrectly into the system.
- Another point that should be kept in mind is that these devices need energy and batteries are heavy.

Deployment Health Surveillance

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ABSTRACT

A major lesson learned from the Gulf War was the need for a comprehensive deployment health surveillance system. Suspected adverse affects from vaccines and other preventive health measures, and unconfirmed exposures to toxic substances have resulted in long term challenges for military leaders and health care professionals. It has been impossible to determine the causes of ill health among Gulf War veterans, in part because of the lack of a comprehensive deployment health surveillance system.

The Department of Defense has responded to this clear need through the development of a force health protection program designed to insure the health of service members before, during and after deployment, and a number of automated systems providing comprehensive medical and environmental surveillance.

These established and emerging surveillance systems continue to evolve as technology advances, continually improving DoD's ability to monitor, and make sound decisions about, the health of service members.

1.0 INTRODUCTION

Good morning. I'm Ellen Embrey, Deputy Assistant Secretary of Defense for Force Health Protection and Readiness. Today I will tell you how the U.S. Department of Defense uses automated systems to help support our force health protection and enhance awareness of deployment health.

1.1 A Layered Force Health Protection System

But first I'd like to discuss the framework we use to accomplish force health protection. Force health protection embodies all the department's policies and programs assigned to provide quality health and medical services to servicemembers and their families throughout their careers. These policies and programs strive to ensure that we recruit and sustain a fit and healthy force, protected from health threats across the full spectrum of military operations, and supported by mobile, technologically advanced clinical teams capable of effectively treating any injuries and illnesses that occur.

Our force health protection priorities are evolving in response to a revolution in military affairs and globalization. Since the 1980s, we have recalibrated our forces to meet the new global threats. Today, we must protect against the not only threats of nation states with massive armies and weapons of mass destruction, but also against the threats of individual terrorist groups as well. We are in the process of

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changing the fundamental basis for military force development – from a threat-based approach, which focuses on who the adversary might be, to a capabilities-based approach, which focuses on countering how the adversary might fight.

If we apply this evolution to military medicine, the focus shifts to a medical force that is capable of not only delivering care anywhere in the world, but also preserving and enhancing the service member's performance in any environment. To accomplish this, effective medical and environmental surveillance become essential, because there is no other way to empirically understand and effectively assess the effects of a combat environment on service member health and performance.

Our force health protection program is designed to optimally protect and maintain the most valuable warfighting assets that the U.S. fields — its soldiers, sailors, airmen, Marines and Coast Guardsmen. Clearly, a fit and healthy force is essential for mission success because they are more resilient and resistant to illness, less prone to injury or the adverse influences of stress, and more likely to rapidly recover from injury or illness.

Our force health protection program depends heavily on automated systems that gather and store data that will inform and support our efforts to preserve force health. This includes environmental and medical surveillance, tracking injuries and accidents, and implementing a deployment health quality assurance program. To understand the significance of these automated systems, you need to understand the three pillars of force health protection, and how they are interrelated.

1.2 The pillars of Force Health Protection

The first pillar of force health protection -- ensuring a healthy and fit force -- focuses on programs that set standards and promote health and fitness force-wide. This is accomplished primarily through prevention and treatment, such as conducting periodic health assessments, assessing medical readiness, running health promotion programs, providing stress management support, and executing a rigorous pre- and post-deployment health screening program.

Our healthcare providers practice preventive medicine, promote healthy lifestyles among their patients, encourage good eating habits, and most importantly, encourage patients to take responsibility for their own long-term health and fitness.

Maintaining a healthy and fit force requires dedication and commitment from every service member and command emphasis on maintaining medical readiness. To that end, commanders' support of health promotion programs is essential. In fact, the presence or absence of command emphasis is the primary reason we succeed or fail in achieving a healthy and fit force.

For example, we rely on commanders' emphasis to achieve our goal of having 95 percent of the force demonstrate excellence in physical fitness by achieving standards of cardiovascular fitness, muscular strength and endurance, flexibility, and agility. We offer service members nutrition education programs and emphasize dental health readiness requirements. We offer substance abuse counselling, maintain a vigorous immunization program for servicemembers, and offer expanded services to deploying forces to better prepare for the stresses of the combat environment. Commanders have also significantly helped us to perform timely and accurate pre- and post-deployment health assessment screenings.

We sustain the health of our forces and their families through the TRICARE health plan, which delivers comprehensive health benefits to 8.3 million servicemembers, retirees and their families, worldwide. In addition to providing the best care possible, the system offers its beneficiaries TRICARE On-Line, a Web site that provides in-depth health education resources, appointment scheduling, and information on military medical facilities and providers.

To better support reserve component service members now eligible for care because of their service in Operations Iraqi Freedom and Operation Enduring Freedom, we are engaged in special efforts to increase access to care and enhance their awareness of health care benefits.

As I mentioned before, we rely on service members to take individual responsibility for their health and fitness. This includes avoidance of unhealthy behaviors like alcohol abuse and cigarette smoking. Respiratory tract infections are among the leading mission-impacting health events in military contingencies, and smoking is associated with an increased incidence of those infections. For example, we had 19 cases of severe pneumonia in Operation Iraqi Freedom. Cigarette smoking was common among all of those patients, indicating that it may have been a contributing factor in the severity of the cases. To reduce this risk, we have been offering smoking cessation programs at every DoD installation.

We are equally concerned about the 80 servicemembers we lose on average each year to alcohol-related accidents. We believe that command emphasis and prevention could dramatically reduce the number of such deaths.

Working together, individual servicemembers, leaders at all levels, and military health care providers can enhance the health and fitness of our forces through individual commitment and full participation in health promotion and fitness programs.

The second pillar of force health protection focuses on programs designed to prevent injury and protect the force against health hazards. We accomplish this through pre-deployment immunizations, countermeasures, and our efforts to maintain safe and healthy working conditions.

Preventing injury includes prevention of casualties from operational, environmental and occupational threats, and protection from biological and chemical threats.

Historically, our forces have suffered more losses from disease and non-battle injury than from enemy action. With 183,000 troops currently deployed to Operation Iraqi Freedom, we have suffered 345 hostile deaths and 155 non-hostile deaths as of January 15th. In Operations Desert Shield and Desert Storm, 235 deaths were caused by accident or illness, and in the Vietnam conflict, more than 10,000 were lost to accidents or illness. Clearly, our recent efforts have been remarkable in reducing the number of deaths due to accident or disease.

The basic principles of disease prevention in the field really haven't changed much. Hand washing, food sanitation, water purification, proper waste disposal and correct use of insect repellents remain essential. And while our vaccinations against potential biological weapons like anthrax and smallpox receive a lot of attention, routine immunizations and prophylactic pharmaceuticals are just as important to prevent endemic diseases.

Comprehensive and ongoing environmental surveillance is also vital to our prevention efforts. By analyzing the air, soil and water, we can potentially avoid environmental contaminants and potentially harmful exposures.

Often the best approach to preventing disease and injury is to use protective equipment. In the most common situations, this means consistent use of earplugs for hearing protection, goggles for vision protection, and the proper application of sunscreen or insect repellent. At the other extreme, it could mean preparing our troops to face the risk of chemical or biological attack with protective suits and masks that provide good visibility and reasonable comfort.

Over the last year, we have also become more focused on ways to prevent and protect against the disabling effects of stress on military life during wartime. Refined and expanded outreach programs offer a variety of venues for servicemembers and their families to receive mental health counseling and family support services to help reduce combat stress casualties.

And just as servicemembers bear the responsibility for maintaining physical fitness, they also contribute much to casualty prevention through measures such as properly wearing protective equipment and uniforms. Individuals must also follow approved procedures for food and water sanitation and waste disposal. Simply washing their hands regularly will help avoid gastrointestinal illnesses. Following safety precautions goes a long way toward injury and illness prevention.

The third pillar of force health protection focuses on DoD's programs that provide world class medical and rehabilitative care anywhere in the world. This includes first-responder care, forward surgery, theater stabilization, en route care, and movement to definitive care. The capabilities in this pillar are changing as advanced and mobile technologies continue to enhance our ability to deliver a lighter, faster, more responsive medical capability in theater.

Our first responders provide field treatment focused on saving life and limb, while additional medical professionals provide essential care to achieve stabilization in theater. Our "critical-care capable" transportation and clinical support teams and systems maintain essential care during all phases of evacuation, supported by improving communication and management systems designed to permit flexible and timely evacuation from theater.

We have reintroduced rehabilitative care as a critical component of this pillar to emphasize our commitment to restoring servicemembers to a state of health that will support their return to duty as soon as possible. Restorative care is an essential feature of good medical care which facilitates a seamless transition to rehabilitative care, if needed, by our clinicians in DoD or within the Department of Veterans Affairs.

The long-term success of our force health protection program requires smart investments in our military health system's infrastructure. Without the ability to plan and execute high-quality military medical training, logistics, information management systems, and cutting-edge research and development, we will not be able to improve, evolve and sustain the quality of our health care system. Ongoing investments in these programs are yielding important force health protection enhancements for our future force. Our medical research, development and delivery programs are being increasingly linked to enhance operational capabilities. We are currently tailoring information systems to satisfy the requirement for smaller, lighter, and more flexible medical forces in theater, while also working to provide real-time decision tools to commanders and other decision makers for assessing ongoing environmental and health risks in theater.

Because our evolving doctrine calls for the delivery of essential care in theater and timely and rapid evacuation to definitive care locations outside the theater of operations, we must continue our efforts to provide an advanced evacuation system that provides rapid movement of casualties from point of injury or illness to point of care. Enhancing the "Golden Hour," or better yet, the "Brass Ten Minutes," through

advanced life saving technology is critical to success, particularly if the future force becomes more dispersed in the battlespace. Future medical capabilities will rely even more heavily on a first responders' ability to provide initial life-saving essential care, while forward resuscitative surgery teams will treat and stabilize casualties prior to evacuation for definitive care.

1.3 Medical Surveillance Systems in Action

Now that you are familiar with the three pillars of force health protection and the infrastructure that is needed to support them, I want to discuss our view that health and environmental surveillance is essential. It offers empirical data to help study health outcomes. It permits us to propose ways to optimize servicemember performance and improve resilience and endurance in different environments and under various operational conditions. Health and environmental surveillance includes the collection and assessment of data on the safety and effectiveness of vaccines and medical pre-treatments, which protect against naturally occurring diseases and chemical, biological, radiological and nuclear threats.

We have learned that necessary vaccines and pre-treatments can cause as much concern as comfort to our servicemembers. To allay those concerns, and to be sure that our forces receive the correct immunizations at the proper time, records of all military vaccinations are now tracked electronically. The Military Vaccine Agency — called MILVAX — maintains surveillance on vaccinations given to counter threats against anthrax and smallpox attacks. And our Defense Medical Surveillance System provides a link between the DoD Serum Repository and other surveillance databases. This repository contains over 30 million frozen serum specimens and is the largest of its kind in the world.

A specific program of medical surveillance has been established to capture health information on deployed service members before they deploy, while they are deployed and upon their immediate redeployment from theater. The Defense Medical Surveillance System maintains this health data. In addition to maintaining health assessment results, this information system contains up-to-date and historical data on reportable diseases, hospitalizations, ambulatory visits, HIV tests, and longitudinal data on personnel and deployments. The department routinely publishes summaries of notifiable diseases, trends of illnesses of special interest and field reports describing outbreaks and case occurrences in the Medical Surveillance Monthly Report, our principal vehicle for disseminating medical surveillance information of broad interest.

In addition to documenting the health status of our deployed servicemembers, we also need to document the location of troops who have illnesses or injuries. Five years ago, we started building a system to track medical evacuations from the theater of operations. That system is now the Transportation Command Regulating and Command & Control Evacuation System. We call it TRAC2ES. Today, TRAC2ES combines transportation, logistics and clinical risk information to enable us to effectively manage patient movement and evacuation anywhere in the world. This system is capable of assessing and prioritizing requirements, assigning proper resources, and distributing relevant data in time to deliver patients to the right facility for the right care. This system also tracks illnesses from the point of occurrence.

One area of medical surveillance that may surprise you is "focused logistics." Our medical logistical services are increasingly applying commercial practices to reduce expensive inventory investments, speed resupply, and more rapidly respond to ever-changing military situations. Similarly, individual medical training has become more intensive, more flexible, and more high tech, from full situational training for medical providers to preventive and first aid training for forces at all levels.

In order to give medical commanders visibility of in-theater assets and capabilities, we developed the joint medical workstation, or J-MeWS. This system, recently fielded in Operation Iraqi Freedom, allows commanders visibility into theater-wide medical command and control operations such as the blood supply in each of the field hospitals, how many empty beds there are at each forward facility, and other vital information. The workstation also serves as the key system for electronically capturing in-theater patient encounter records, as well as disease and non-battle injury data.

As effective as the automation systems I just described are, these will ultimately be subsumed by implementation of more capable DoD-wide standard systems. Let me give you a brief view of the future. J-MeWS is the leading edge of what will soon grow into the Theater Medical Information Program, or TMIP. The objective with TMIP is to provide a theater-based medical information system fully interoperable with and utilizing the same data repository as all medical facilities within the department worldwide. TMIP's integrated medical information systems will ensure precise, interoperable support for rapid mobilization, deployment, and sustainment of all theater medical services anywhere, anytime, in support of any mission. Through TMIP's medical surveillance system, theater commanders will gain situational awareness for critical decision-making. Commanders will be able to track trends, take preventive actions, and keep their forces fit through a heightened new ability to collect, analyze, and make use of collective medical information across the Services throughout the theater in near real-time. They will be able to determine the location and health status of injured warfighters across the theater and the types and skills of replacement personnel required. Needless to say, TMIP will provide the information needed to support force health protection in the theater.

As a complement to medical surveillance, vigilant environmental surveillance helps us quickly identify and quantify levels of chemical, biological, radiological, nuclear, occupational or environmental exposures. Such surveillance provides empirical data needed to assess health risk resulting from various contaminant levels in food, water, air and industrial chemicals.

Today the Department, primarily through the great work of the U.S. Army Center for Health Promotion and Preventive Medicine, conducts environmental surveillance by monitoring the air, water and soil where servicemembers are deployed overseas. This allows us to avoid areas that may pose more serious health threats due to industrial pollution or endemic diseases. By further automating this activity, we can get a better historical view of health effects as they relate to troops in a particular area. That is why we are developing the Defense Occupational and Environmental Health Readiness System, or DOEHRS. This automated information system will provide timely and efficient access to data and information for DoD users throughout the world. When completed, the system will capture data on environmental and occupational exposures for transfer to a computerized patient record. While deployment exposure data isn't being fed into DOEHRS yet, the system already supports data relating to our hearing conservation and industrial Hygiene programs within the Military Health System. Ultimately, DOEHRS will enhance readiness by providing information to enable exposure-based medical surveillance and enhanced industrial hygiene risk management, and will enhance our ability to provide occupational health care and wellness programs for the Department of Defense workforce.

Medical surveillance isn't solely for the protection of deployed servicemembers. As I said at the beginning, the number one pillar of force health protection is to maintain a fit and healthy force. That's why we're so proud of our newly published standards for achieving individual medical readiness. To assure adherence to these new standards, a new automation tool is emerging. Currently the Services maintain separate systems for this data, but they are now using a common set of individual medical readiness standards to monitor the collective readiness of the force. For a servicemember to be fully medically ready, all immunizations must be current and any important dental work must be done. They must have all medical readiness lab tests completed, including HIV tests, have no deployment-limiting medical conditions, have completed a current

health assessment, and have all the medical equipment they need, including ear plugs, eyeglasses and mask inserts. The individual medical readiness standards are less than a year old, so there's no prior data to compare yet. However, we plan to have the databases populated by the end of this fiscal year. By tracking compliance to these individual medical readiness standards, commanders will be able to monitor medical readiness and will be able to take specific actions to improve it. This is an important new commander's tool.

If this sounds like a narrow focus, let me give you an example of a broader perspective on these health issues. The DoD Global Emerging Infections Surveillance and Response System — DoD-GEIS — is designed to strengthen the prevention of, surveillance of and response to infectious diseases that could threaten military personnel and their families. DoD-GEIS creates a centralized coordination and communication hub to help organize DoD resources and link with U.S. and international efforts. This system leverages the surveillance and response assets of a network of DoD service hubs and overseas medical research units. It gives us a global view of the presence and movements of diseases.

DoD-GEIS brings with it a new prototype system called ESSENCE: Electronic Surveillance System for the Early Notification of Community-based Epidemics. This system is specifically geared to early detection of infectious disease outbreaks at military treatment facilities. It provides for surveillance of syndromes recorded at the time of patient visit instead of specific diagnoses reported after laboratory or other diagnostic procedures. This can greatly lessen the time it takes to determine that an outbreak is occurring. Using historical data, a prediction of normal ranges can be performed. Our current ESSENCE version prepares a list of the most aberrant down to less aberrant sites whose syndrome-specific incidences exceed the modeled expectation. These "suspicious" data are hyperlinked to show relevant graphical trends for expert surveillance analysis and response actions, as appropriate.

A similar system developed by the Pentagon is called LEADERS – the Lightweight Epidemiology Advanced Detection and Emergency Response System. LEADERS isn't just a system for military leaders. Public health officials, emergency management agencies, hospitals and physicians can also access LEADERS over secure phone lines or satellite connections using a Web browser to determine if there is a potential health risk in a community. Hospitals enter symptom and abnormalities data into Web collection forms to determine if a particular syndrome needs to be tracked. The system then analyzes the medical data to spot trends. If the system finds an unusual trend it posts a warning on the alert screen. Health officials then analyze alert details. Doctors can transmit the information to the federal Centers for Disease Control and Prevention in Atlanta to determine if there is a public health emergency.

The next big step in force health protection automation systems will be to erase the line between deployed and non-deployed medical surveillance. We can accomplish that with the Composite Health Care System. The CHCS was conceived as a fully integrated automated medical information system for Department of Defense health care facilities worldwide. It automates inpatient and outpatient medical information in patient administration, patient appointment and scheduling, radiology, pharmacy, laboratory, nursing and clinical services management. The system has already evolved into CHCS II, a medical and dental clinical information system that will generate and maintain a comprehensive, life-long, computer-based patient record for each Military Health System beneficiary. CHCS II was designed to meet the challenge of making medical and dental records immediately available to providers caring for a highly mobile population that includes 1.4 million active duty Armed Service members around the world. The system provides access to a beneficiary's comprehensive health record, which includes data on preventative care, illnesses, injuries, and exposures treated at any military treatment facility. In concert with TMIP, DOEHRS and the other systems I've mentioned today, CHCS II will eventually be the foundation of a universal health surveillance system, supporting both deployment health policy and force health protection programs.

The future of force health protection will rely on persistent detection of threats with an integrated and shared view of the battlespace, and on timely dissemination of accurate warnings, risk assessments and decisions which will allow the force to protect itself against specific attacks and threats. Force health protection must be proactive, focused, and conducted by integrated military forces.

Automated health surveillance capabilities will soon enable rapid collection and analysis of data on diseases, battle injuries and non-battle injuries, including combat stress. This information will be available as a by-product of capturing patient encounter data in an electronic medical record.

Eventually, we expect to be able to use these automated medical records to help us correlate relationships between an individual's deployment experience and any medical problems. Perhaps with well-documented electronic health and environmental surveillance systems, we can begin to understand the health impacts of low-level exposures to various toxic substances and take appropriate actions to prevent or minimize such exposures.

Rapid and effective medical treatments of the future must come through joint capabilities that are light, agile, interchangeable, and interoperable. We'll need to be able to support highly mobile and dispersed joint forces rapidly projected anywhere on the globe. It is imperative we provide the best support possible to our most important weapon of all: the men and women of our Armed Forces.

We have the technology to meet most of our force health protection requirements, and other technical solutions for the remaining requirements are on the horizon. It has been an honor and a pleasure to be here today to discuss these important topics. Thank you.

Technology Opportunities: Implementation of Deployment Health Policy in Operational Theaters

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ABSTRACT

It is U.S. policy that medical and personnel information systems be designed, integrated, and utilized with military medical surveillance to protect the physical and mental health of Service members throughout their military service. Within the last several years, new applications of information technology have vastly expanded our capabilities for surveillance, and these technologies are now starting to move out of hospitals and other fixed facilities into forward deployed settings. Surveillance technology must evolve toward an integrated system of systems that can comprehensively address future needs to identify acute and chronic exposures of military personnel to health threats over the course of their entire military career. Many of the necessary component technologies are available now or will be soon - the key will be to integrate them.

1.0 INTRODUCTION

In accordance with Department of Defense Directive dated August 30, 1997, it is the policy of the United States Armed Forces that medical and personnel information systems be designed, integrated, and utilized with military medical surveillance to protect the physical and mental health of Service members throughout their military service. These systems will be continuously in effect and be specifically configured to assess the effects of deployment on the health of Service members by encompassing the periods before, during, and after deployment.

Medical surveillance is the routine, systematic collection, analysis, interpretation, and reporting of standardized, population-based data for characterization of and countering medical threats to population health, well-being, and performance. It consists of active, passive, and sentinel procedures. Deployment health surveillance includes identifying the population at risk through personnel unit databases as well as pre-deployment and post-deployment health assessments, recognizing and assessing potentially hazardous occupational and environmental health exposures and conditions, employing specific preventive countermeasures, monitoring real-time health outcomes, and reporting disease and non-battle injury (DNBI) data to higher headquarters in a timely manner.

Medical surveillance requires an understanding of the complex inter-relationship of the environment, threat agent, and at-risk host. The types of threats include those from both environmental sources and intentional attack, and the consequences from either may result in acute illness, chronic illness, or both. A successful

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surveillance program requires aggressive data collection including environmental monitoring (vectors, water, soil, climate, air, noise, food, and other environmental sources), data analysis, feedback, and countermeasure actions including the required modifications to data collection. Together these actions comprise the Surveillance Cycle.

The incentive for developing a comprehensive surveillance program is substantial. DNBIs represent the largest proportion of morbidity and mortality in deployed forces. A successful program will assist commanders in assessing the impact that diseases and injuries are having on the availability for duty of individuals and entire units. In addition, early detection of diseases, risks, and hazards will allow commanders to complete an operational risk assessment matrix and employ appropriate countermeasures, thus multiplying the force and conserving health care resources.

Within the last several years, new applications of information technology have vastly expanded our capabilities for surveillance, and these technologies are now starting to move out of hospitals and other fixed facilities into forward deployed settings. We are rapidly approaching the time when we can truly say that we have a fully integrated and comprehensive system for conducting effective surveillance in operational theaters.

2.0 SURVEILLANCE PROGRAMS AND SYSTEMS

A successful surveillance program requires a "system of systems" that makes full use of available technologies. A key factor is that systems need to be connected in "real-time" to support every aspect of the Surveillance Cycle and provide for force health protection. Several technology applications have been developed that are already, or could become, components of this system of systems. Some of these applications have already been integrated, while others are simply prototypes or early concepts. The following discussion highlights the capabilities of current surveillance programs and the technology applications that are included within them.

2.1 Department of Defense Global Emerging Infections Surveillance and Response System (GEIS)

The GEIS brings together laboratory partners from 58 countries and several agencies and organizations including the World Health Organization and the Centers for Disease Control and Prevention (CDC) to identify new disease events. The surveillance priorities include respiratory illness (especially influenza), febrile illnesses, enteric illnesses, and antimicrobial resistance. In addition to global surveillance, GEIS assists the global response to a new disease event by providing uniquely capable, permanent, multidisciplinary platforms for staging and supporting a wide range of field, laboratory, human, and veterinary health investigations. GEIS also assists in building global capacity, thus creating focal points for leveraging local capacity through training and infrastructure strengthening. The GEIS has over 30 influenza surveillance sentinel sites located worldwide (Figure 1). A key part of the GEIS is the Early Warning Outbreak Recognition System (EWORS) that was developed by the U.S. Navy and the Indonesian Ministry of Health. EWORS is a computerized hospital-based surveillance network for early detection of outbreaks. EWORS monitors trends to differentiate an epidemic from endemic disease events.

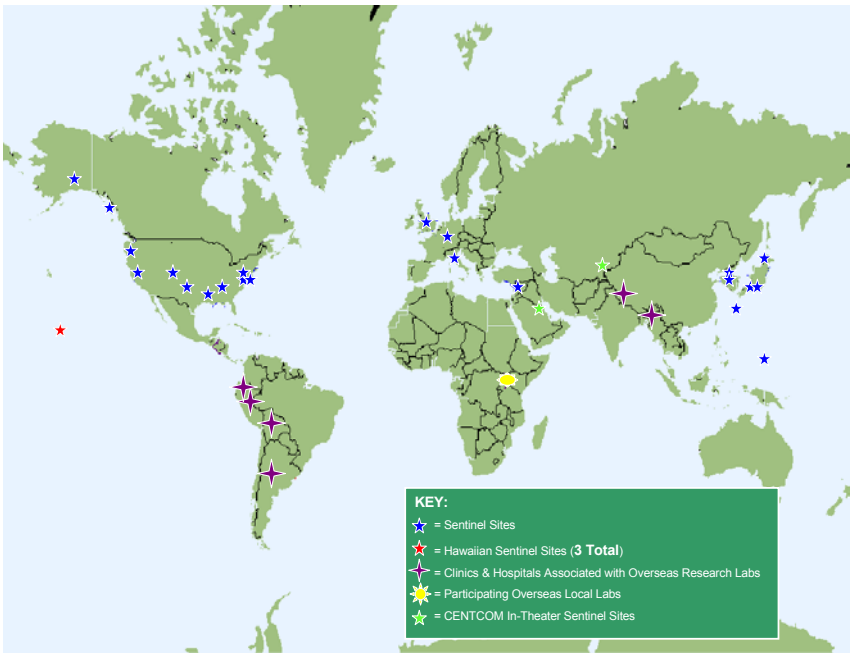

Figure 1: DoD GEIS 2003–2004 influenza surveillance sentinel sites

2.2 Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE)

ESSENCE is a technology application that is an outgrowth of the GEIS. ESSENCE is designed to facilitate syndromic surveillance as an adjunct to traditional reportable disease surveillance and sentinel systems. It gathers information regarding outpatient medical visits for detection of potential infectious disease outbreaks and monitoring of disease trends. The system was developed and is run by the Walter Reed Army Institute of Research in collaboration with the Johns Hopkins University Applied Physics Laboratory. ESSENCE can identify increased visits for broad infectious disease categories (Figure 2), which may herald release of a biological agent. Most fixed military treatment facilities (there are only a few exceptions) are included in the surveillance system. The system is available on a secure password-protected website, and no patient identifying information is presented. ESSENCE monitors conditions using International Classification of Diseases, 9th Revision (ICD-9) codes entered into the Ambulatory Data Module and Standard Ambulatory Data Record as follows:

- Respiratory (cough, pneumonia, and upper respiratory illness)
- Gastrointestinal (vomiting and diarrhea)
- Neurologic (meningitis and botulism-like)
- Dermatologic – hemorrhagic (petechaie and bruising)
- Dermatologic – infectious (vesicular rashes and smallpox-like)
- Fever/Malaise/Sepsis (unspecific fever and sepsis)
- Coma/Sudden Death (coma and sudden death)

In addition, ESSENCE monitors the percentage of influenza-like illness (ILI) seen at primary care clinics, similar to the CDC sentinel influenza surveillance. An advanced version of this system (ESSENCE IV), which includes pharmacy data and runs more advanced statistical algorithms and geographic analysis, is in development.

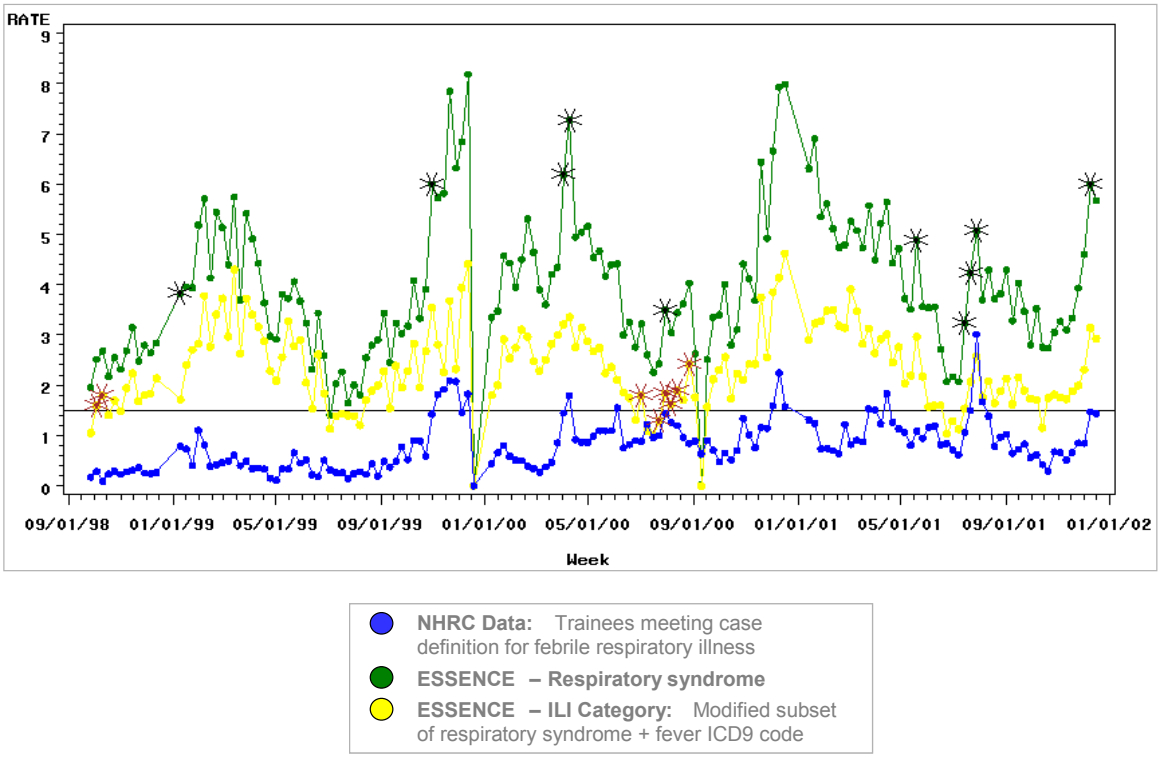

Figure 2: Monitoring of respiratory illnesses, comparing ESSENCE respiratory and ILI groups to a traditional surveillance system, the Naval Health Research Center (NHRC) febrile respiratory illness surveillance, which monitors the number of basic trainees who fit a clinical case definition per week

2.3 Defense Medical Surveillance System (DMSS)

DMSS is a database system for routine and systematic collection of longitudinal data and thus provides medical surveillance decision support. The DMSS is maintained by the Army Medical Surveillance Activity at the Center for Health Promotion and Preventive Medicine (CHPPM) and stores data on active duty service members from pre-induction through post-discharge. The DMSS serves as the central repository of medical surveillance data for the U.S. Armed Forces and includes personnel data, medical data from ambulatory care and in-patient care records, immunization records, deployment records, and data from pre- and post-deployment health assessments (Figure 3). The DMSS includes the Reportable Medical Events System (RMES). RMES uses dedicated reporting software to track and report 70 specified medical conditions. It automatically transmits the data to the main database of the DMSS and issues summary reports as feedback. RMES data support the investigation of unusual events.

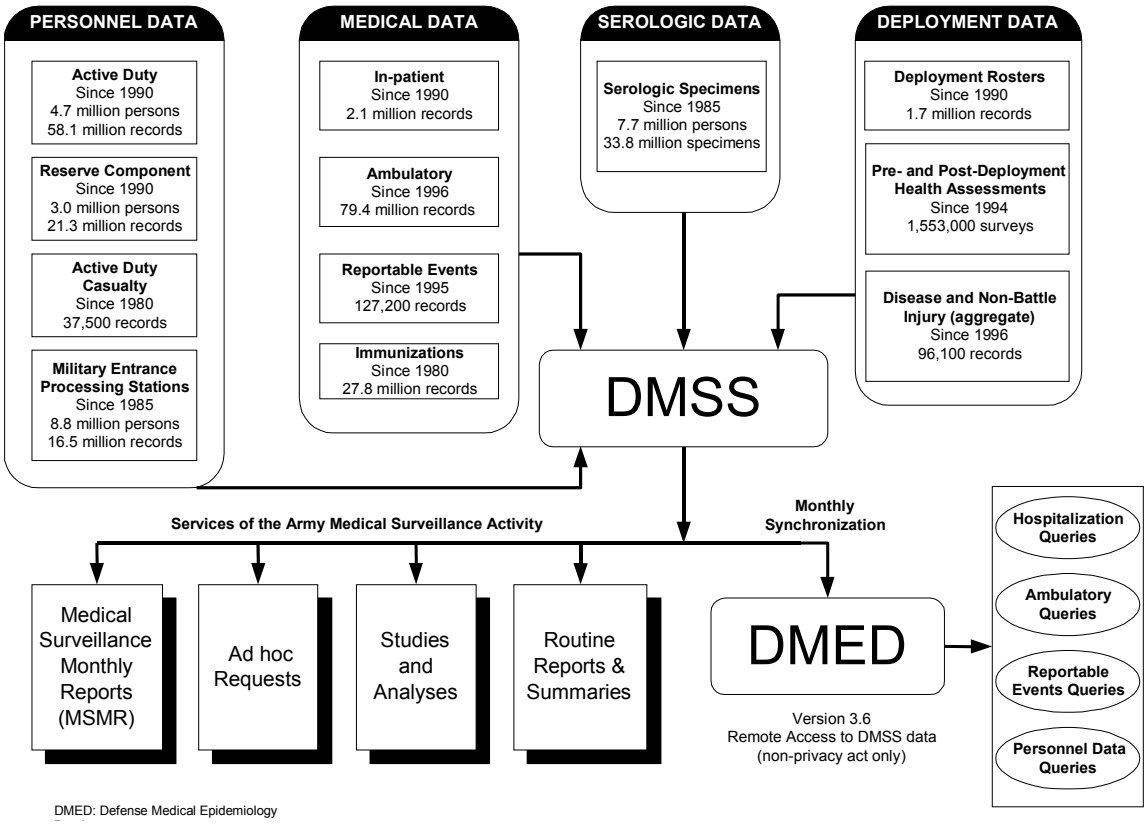

Figure 3: DMSS structure and functional relationships

2.4 Department of Defense Serum Repository (DoDSR)

The DoDSR is the central archive of sera drawn from U.S. Armed Forces personnel for medical surveillance purposes. The repository contains over 3.3 million serum specimens from over 7.7 million personnel, and it has a capacity to hold 40 million specimens. The sera are stored at precisely documented locations in large walk-in freezers held at –30ºC, and all specimens are linked to demographic, military, and medical information via the DMSS. Specimens contained in the DoDSR are available to researchers and other investigators within the DoD for the purposes of conducting militarily relevant investigations.

2.5 Defense Occupational and Environmental Health Readiness System (DOEHRS)

The DOEHRS is the occupational health migration system for the DoD. It consists of an automated system designed to support the programs of Industrial Hygiene, Environmental Health, Hearing Conservation, and Occupational Medicine. It serves as an operational data store and data repository, and it produces periodic reports, answers to ad hoc queries, and trend analysis.

2.6 Deployment Environmental Surveillance System

Initially created to maintain environmental surveillance data for Operation Joint Endeavor, the Deployment Environmental Surveillance System is the central information system for the CHPPM Deployment Environmental Surveillance Program (DESP), and is integrated with the Laboratory Information Management System at the CHPPM. The system serves as a project management tool, provides for a standardized collection format, manages and validates field data, manages and validates analytical data, and serves as a data repository. Ultimately, the system is designed to be integrated with the Industrial Hygiene module of the DOEHRS.

2.7 Emerging Geographic Information System (GIS)-Based Applications

GIS refers broadly to a class of computer-based systems that are used to store, create, and manipulate geographic information. It expands the use of traditional paper maps, particularly by overlaying diverse data layers. GIS enables the user to answer geographical health-related questions and reveal relationships, patterns, and trends. The background data can be obtained from a wide variety of sources including government assets and commercial sources; however, satellite imagery is one of the most powerful tools. The background data are overlaid with specific military data such as troop locations, infrastructure information, situation reports, engineer data, and operational deployment orders. The resulting solution is a map that shows the locations of hazards in operational areas.

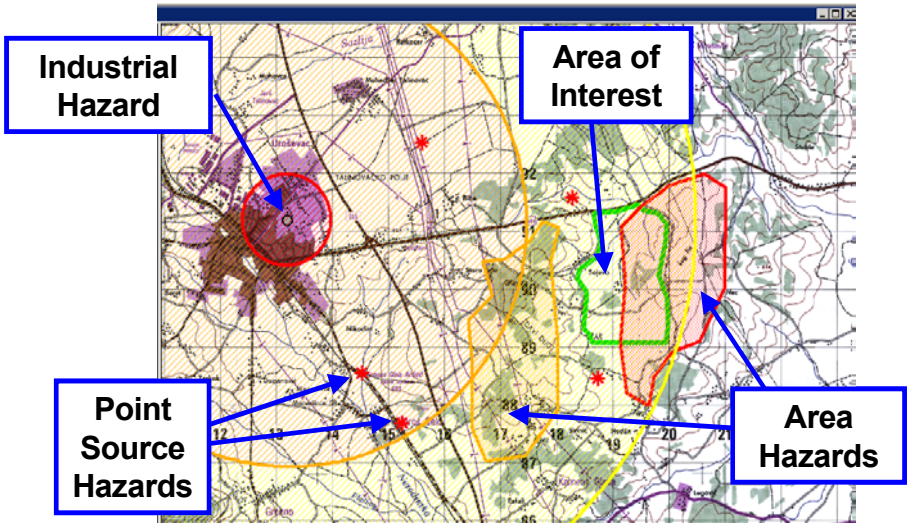

Figure 4: Use of GIS technology for chemical hazard risk analysis

GIS has already been used for assessment of chemical and disease risks. As one example, GIS can be used to identify a variety of local chemical hazards and associated risks prior to deployment, so that such areas can be avoided or exposure risks can be mitigated in other manners (Figure 4). GIS may also be used for spatial analysis for casualty planning. For example, one could use modeling of case-specific conditions (chemical release or meteorological conditions) to determine the potential impact from an accidental or intentional release of a chemical. GIS may also be used for spatial and temporal analysis to track the change of conditions over time and to direct sampling efforts in the affected area. GIS is also being used in combination with thermal imaging and disease vector surveillance techniques to identify areas of potential high vector concentration (Figure 5). The ability to model disease outbreaks based on satellite imagery that provides an indication of the local ecosystem is emerging. Recent studies have shown, for example, that Ebola outbreaks appear to be preceded by local drought conditions and can be predicted by analysis of satellite images that measure the color of the forest canopy.

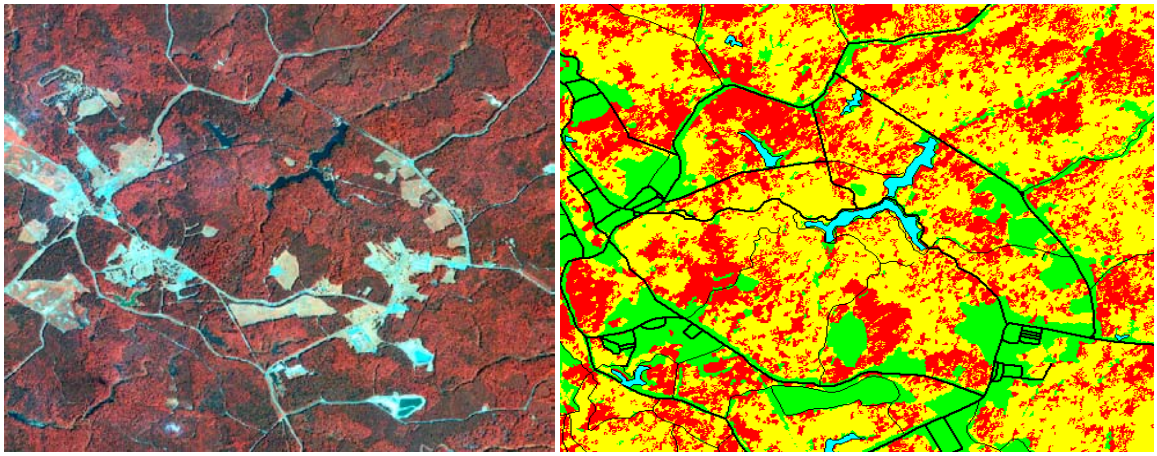

Figure 5: Use of GIS technology for disease vector analysis. Left image is an IKONOS satellite image with near infrared band of the study area. This type of satellite image allows identification of different vegetation types and is used to characterize tick habitat. The right image is a theoretical threat assessment of a map of the same area showing the probability of encountering lone star ticks that spread Lyme disease. The satellite image has been "classified" into three categories representing the likelihood of encountering lone star ticks, with red, yellow, and green representing high, moderate, and low probabilities, respectively, of encounter.

3.0 FUTURE SYSTEMS

New technologies are emerging that will push surveillance capabilities even farther forward. This will be made possible by the miniaturization of computing capabilities and new sensor technologies, as well as by the ability to connect these systems to the larger surveillance system of systems.

3.1 Battlefield Medical Information System-Tactical (BMIS-T)

The BMIS-T is an example of these emerging capabilities. It is a point-of-care handheld assistant that enables military providers to record, store, retrieve, and transmit the essential elements of patient encounters in an operational setting (Figure 6). Reference materials, diagnostic and treatment decision aids, and logistic support software can also be included, thus facilitating patient care, skill sustainment training, and mission planning. Initially designed for Special Forces medics and other first responders, BMIS-T can be used by

providers at all echelons along the health care continuum. BMIS-T gives medical providers an all-in-one tool for medical readiness, clinical information capture, diagnosis, and logistics to improve patient care and record keeping and contribute to a better informed, more effective military force. By streamlining data capture and reporting, BMIS-T helps to ensure more complete patient records. As medical practice evolves, BMIS-T has the flexibility to incorporate new procedures, protocols, medical databases, and mission requirements. The BMIS-T provides data from forward encounters into the Composite Health Care System II - Theater (CHCSII-T). CHCSII-T is a clinical operations data system designed for Level I to Level III treatment facilities in operational settings. Although not intended as a primary surveillance system, it supplies raw data for surveillance inquiries.

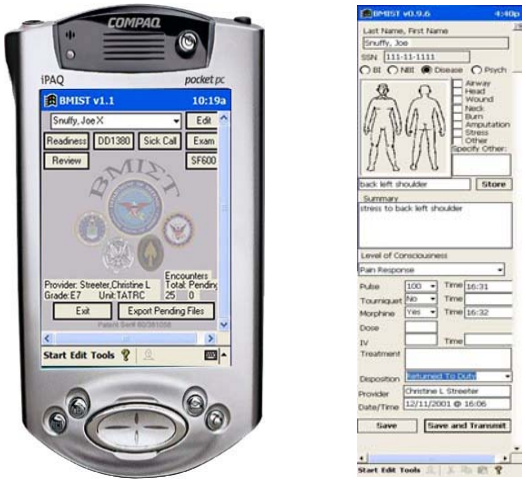

Figure 6: The BMIS-T, shown at left, is a handheld system that will allow capture of medical encounter information by far-forward medics. A sample display screen is shown at right.

3.2 Individual Status Monitoring

Looking still further into the future, technologies are emerging for collection of near-real-time information on individuals' exposure to toxic, disease, and other health threats. Work is currently being undertaken to understand the genomic response to various health threats and identify diagnostic patterns. Once this information is in hand, it is possible to envision forward deployable gene chip-based systems that will provide early warning of disease outbreaks and ability to assess toxic hazard exposures. Such systems would still require a sample to be drawn from an individual and so require some type of encounter with the health care system. Ultimately, however, sensor technologies are expected to evolve to the point where useful information can be obtained in near-real-time from soldier-worn sensors. The Warfighter Physiological Status Monitor (WPSM) is an overarching concept for a series of technology insertions into the soldier's individual ensemble. In the near-term, WPSM is being designed to monitor sleep status, thermal state, and hydration. However, mid-term and far-term goals include the integration of ability to not only detect exposures to toxic chemicals and biohazards, but also assess their biological significance.

3.3 Joint Medical Workstation (JMeWS)

The JMeWS is an example of a system that brings all of the forward-deployed surveillance assets to a focus (Figure 7). The JMeWS is an integrated system composed of the Mobile Medical Data Store database and client/server data input and viewing applications. It utilizes Oracle Enhanced Security Module 3DES encryption and Secure Socket Layer for secure storage and access to the database. The reporting module is a package of custom-designed data entry forms composed of the Annex Q and patient reports. The Annex Q includes medical situation and blood reports. The patient reports document patient treatment and provide patient movement and visibility information. The MDSS is an information and decision support system for medical planning staffs and operational commanders based on advanced data analysis methods for predicting and implementing expedited preventive health measures. The MDSS imports patient encounter data from the JMeWS database, analyzes the data for DNBI incidence rates and trends, and displays the data for medical surveillance alerting and reporting functions.

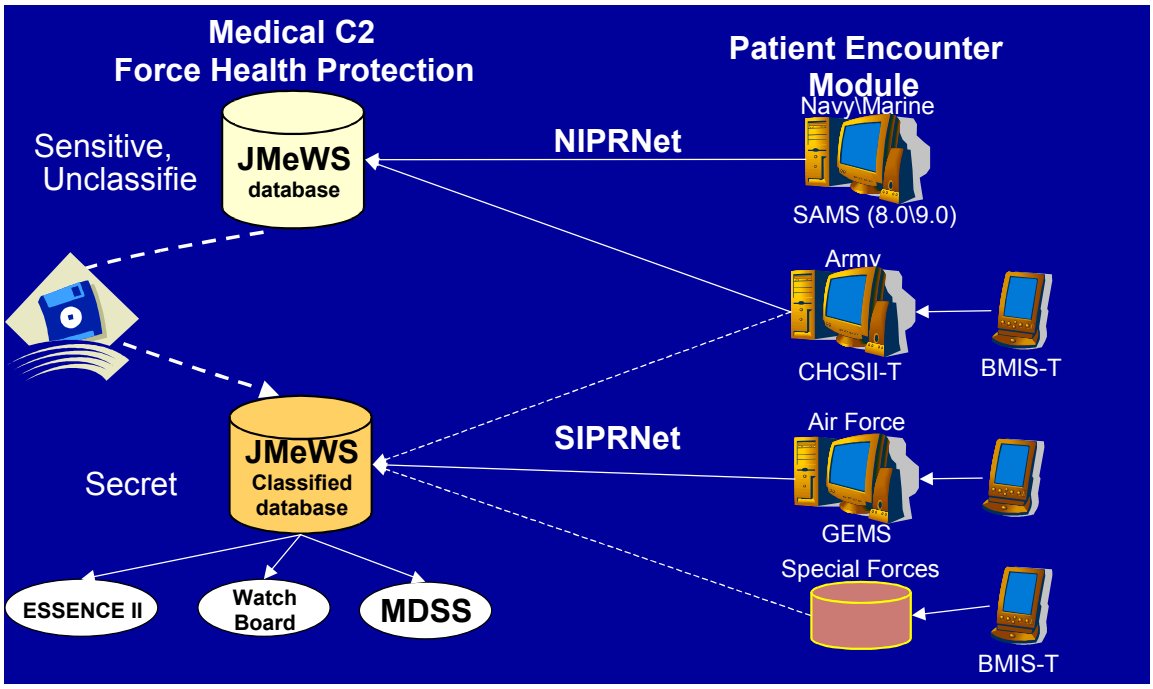

Figure 7: JMeWS, functional linkages to patient information and surveillance systems

4.0 SUMMARY AND CONCLUSIONS

A comprehensive deployment health surveillance program is required to assist the commander in assessing the impact that DNBIs are having on the ability of deployed forces to accomplish the mission and employing appropriate countermeasures as a force multiplier. The program must identify both acute and chronic health risks from environmental threats as well as intentional attacks with biological, chemical, or radiological weapons. To make surveillance systems smaller and deployable farther forward, one must embrace new technologies and conduct active research into and development of more powerful tools. Deployment health data are an important component of a force health protection life cycle that begins pre-accession and extends continuously to post-separation or retirement. A successful deployment health surveillance program depends

on trained personnel that employ active, passive, and sentinel procedures and have access to technological tools and systems that communicate and interact. Many of the necessary component technologies are available now or will be soon - the key will be integrating them to provide visibility of events throughout the soldier's total life cycle. These tools form a "system of systems" that serves to protect the force and support the accomplishment of the mission.

Global Influenza Surveillance in the U.S. Military

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ABSTRACT

Given that infections caused by various emerging respiratory pathogens and known biological warfare agents often present initially as influenza-like illnesses, there is interest in a near-real-time system that can identify both covert attacks involving biological agents and emerging respiratory pathogens. This article describes an approach integrating the results from lab-based influenza surveillance with several complementary health data streams (outpatient, inpatient, immunization, etc.) to create such an enhanced surveillance system.

1.0 INTRODUCTION

The 1918 influenza pandemic serves as a poignant reminder of how devastating influenza can be. Its ability to generate enormous numbers of casualties, probable travel restrictions, and effects on civilian support infrastructure poses a serious threat to military operations. More recently, in 1996, a United States ship of the line was taken out of service and forced into a foreign port for 2 days while waiting for enough crew members to recover before resuming normal operations.[1] In light of this threat, the U.S. Department of Defense (DoD) maintains a globe-girdling influenza surveillance system, seeking to identify antigenic shifts and drifts at the earliest possible moment. Frighteningly, the threat is not limited to influenza. Emerging infectious diseases such as the Severe Acute Respiratory Syndrome coronavirus and, possibly, biological warfare agents loom ahead. The early stages of many of these infections resemble influenza and are often categorized as influenza-like illnesses (ILI). Given these threats, there has been a concerted effort to adapt existing surveillance systems to provide near-real-time surveillance that could identify covert attacks involving biological agents or the emergence of new respiratory pathogens as well as improve the DoD's capabilities to monitor naturally occurring influenza.

2.0 HISTORY OF INFLUENZA SURVEILLANCE IN THE U.S. MILITARY

Systematic influenza surveillance in the DoD began in 1976 by direction of the USAF Surgeon General. It was called "Project Gargle" to remind people to focus on obtaining throat samples from individuals with ILI. Later, the program expanded to include all of the DoD as part of the Global Emerging Infections Surveillance and Response System (GEIS)[2] network. The overall program objectives are to:

- Prevent outbreaks from newly emerging viral strains
- Isolate and identify circulating influenza viruses

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- Detect newly emerging subtypes or antigenic drift
- Evaluate influenza vaccine effectiveness

3.0 METHOD

3.1 Population-based Influenza Surveillance

There are two primary components to the DoD Global Influenza Surveillance program. One is a population-based element while the other is etiologically-focused (see 3.2 below). The population-based element, managed by the Naval Health Research Center, San Diego, California, tracks febrile respiratory illness (FRI) incidence rates among basic military trainees[3]. Trainees merit special attention given their increased risk for respiratory disease outbreaks due to close living conditions, marked physical and psychological stress, and difficulty maintaining personal hygiene under austere training conditions. As a closely monitored and controlled group, they are ideal for surveillance of this type. Medical staff members systematically collect viral specimens (oropharyngeal or nasopharyngeal swabs) from trainees who meet the established case definition. Candidates are those who present for care within 72 hours of symptom onset, have a temperature of more than 100.5º F (38º C), and either a cough, sore throat, or radiographic evidence of a viral pneumonia. These samples then undergo analysis against a viral panel that includes influenza A and B, parainfluenza viruses, respiratory syncytial virus, herpes simplex virus, and adenovirus. This year-round surveillance provides valuable information about current etiologic agents among trainees that in turn helps preventive medicine and public health staff to effectively target their efforts. As the trainees receive a number of vaccines in their first week of training, there is also an opportunity to gather information about vaccine effectiveness.

3.2 Etiologic-based Influenza Surveillance

Etiologic-based surveillance[4], combined with the population-based component described above, completes the picture. Using the same case definition, the DoD medical staff members at 27 sentinel sites around the globe collect at least six samples each per week during the influenza season (October through April) and submit them to the Air Force Institute for Operational Health (AFIOH)[5]. The sentinel sites are carefully selected to maximize the possibility of capturing specimens of newly emerging viral strains. Thus, a number of the sites are located along the Asia-Pacific Rim since many new strains of influenza have emerged from China in the past. Other sites are major military transportation hubs through which service members routinely pass when returning from duties overseas. Nonsentinel military installations and various DoD overseas research laboratories also submit specimens, especially if there appears to be a respiratory disease outbreak in their area. The Air Force Clinical Reference Laboratory at AFIOH analyzes the samples using the same viral panel described in 3.1. Other DoD laboratories, such as the Naval Health Research Center and US Army Medical Centers, are available to help analyze samples during exceptionally busy seasons. Antigenic subtyping and molecular analysis are performed on a subset of the positive samples. Samples and results are shared with the Centers for Disease Control and Prevention (CDC), who in turn collaborates with the World Health Organization (WHO). This information is a critical component in the decision-making process to determine which strains should be included in the next vaccine, and on some occasions the DoD influenza surveillance program has provided the samples best suited for use as seed viruses in the production process.

4.0 ADAPTING SYSTEMS TO DEAL WITH EMERGING THREATS

Emerging infectious diseases are communicable diseases that have recently become more prevalent or threaten to do so. They can include human, animal, and plant infections. They may be the product of a natural disease outbreak or the result of an attack involving biological agents. Recent examples include Severe Acute Respiratory Syndrome, pulmonary anthrax, and avian flu. It isn't possible to create new surveillance systems for each new disease agent, but it is possible to adapt existing systems. In this case, the goal was to adapt and combine existing systems to provide an enhanced respiratory surveillance program. Available DoD systems included the Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE), the inpatient data registry, reportable medical events systems, the immunizations databases, trainee FRI surveillance program, and the laboratory influenza surveillance program. Table 1 provides a synopsis of these systems.

DoD System	Description/ Comments
ESSENCE (Electronic Surveillance System for the Early Notification of Community-based Epidemics)	Syndromic surveillance system monitoring categories of outpatient ICD-9 diagnostic codes from primary care clinics and emergency rooms; central DoD repository, updated 1 to 3 times a day
Febrile Respiratory Illness Surveillance	Population-based sampling limited to stateside basic trainees
Influenza Laboratory Surveillance	Convenience sampling at selected sentinel sites; gold standard laboratory confirmation techniques
Inpatient Data Registry	Discharge diagnoses for all hospitalizations; severity-biased with a 30-60 day time lag
Reportable Medical Events	Limited set of selected diagnoses; passive reporting system; only a subset are urgently reportable (within 24 hours), others lag 30-60 days before reaching central DoD repository
Immunizations Data Registry	All immunizations for military and family members; separate systems in each service, but completeness varies, especially with regard to family members

Table 1: Candidate DoD Systems for Use in Enhanced Respiratory Disease Surveillance

4.1 Specific Actions

Given the various shortfalls, no single system in Table 1 could hope to consistently identify emerging outbreaks at the earliest possible moment. Specific enhancements were made to several of the systems to compensate for these limitations.

4.1.1 Modifications to ESSENCE

The core aspect of the DoD ESSENCE is to monitor ambulatory (outpatient) health event data from selected primary care clinics (family practice, pediatrics, and internal medicine), acute care clinics, and emergency

rooms on a daily basis. The system includes data from all of the permanent U.S. military medical treatment facilities around the world, but it does not encompass deployed medical operations. The ICD-9 diagnostic codes were originally mapped against 7 syndromic categories (Respiratory, Fever, Gastrointestinal, Dermatological-Haemorrhagic, Dermatological-Infectious, Neurological, and Coma). The counts of these categories are compared with historical data with alert (between 2 and 3 standard deviations) and alarm (greater than 3 standard deviations) statistical thresholds that trigger investigations. The original system captured influenza illness in the Respiratory and Fever categories. However, the Respiratory category is extremely broad with over 150 ICD-9 codes. Unfortunately, a small number of unusual cases were easily lost against the year-round high background levels of upper respiratory illnesses. To improve the system's ability to capture variations in flu-like illnesses, a new category for Influenza-like Illness (ILI) was created. This category captures cases from the following areas:

- Viral infection
- Acute pharyngitis, laryngitis, tracheitis (or combinations)
- URI of multiple or unspecified sites
- Acute bronchitis, bronchiolitis
- Viral pneumonia
- Influenza-specific diagnoses
- Nonspecific symptoms (fever, throat pain, cough)

4.1.2 Modifications to the Laboratory Influenza Surveillance Program

The primary modification here was to expand the number and placement of sentinel sites. For the first time, the DoD was able to collect viral specimens from American soldiers supporting Operations IRAQI FREEDOM and ENDURING FREEDOM at their deployed locations. This provided samples from geographic areas where previously access had been quite difficult and yet were places where there was a good likelihood of capturing new strains, such as Central Asia. Besides adding new sites, the laboratories increased their capacity and added automatic sequencing equipment to handle the increased sample load. Another enhancement was to compare each positive USAF influenza sample with the USAF immunization database to determine if the individuals had been vaccinated at least 2 weeks prior to the onset of their illness. If so, they were potential breakthrough cases, and this data could be used to focus efforts on possible drift strains that are not good matches with the current vaccine components. Finally, analysis and reporting were performed more frequently, going from weekly to daily during the periods of greatest concern, e.g., during the peak of SARS cases.

4.1.3 Analysis and Interpretation (Bringing the Pieces Together)

The following scenario occurred on a DoD military installation in the fall of 2003 and illustrates how these complementary data sets were used to better assess both the health and operational risks. During the last week of October 2003, the local medical staff began to notice increasing numbers of respiratory disease cases among technical students attending a local course. There were several cases of relatively severe disease that required hospitalization, but not intensive care. Figure 1 shows the ESSENCE Respiratory graph for this time period. Although there is a general upward trend beginning in late October, a statistical threshold was not crossed until 6 November, and then only at the yellow (2 standard deviation) level. Figure 2 shows the Fever curve for the same time period. Here there is a clear cluster of elevations at the red (3 standard deviation) alarm level in the first week of November. Similarly, the percent of all ambulatory visits due to influenza-like

illness jumps precipitously, as shown in Figure 3. Not shown, but also important, was the laboratory influenza surveillance data for samples submitted from sentinel sites in proximity to this installation. These results were overwhelmingly positive for influenza A/Fujian. Although analysts might have been willing to accept the ESSENCE Respiratory category data as a mild seasonal variant, looking at the other data sets convinced them that there was a significant outbreak underway. An investigative team was dispatched and the annual immunization program accelerated to include the remaining base personnel while the outbreak subsided.

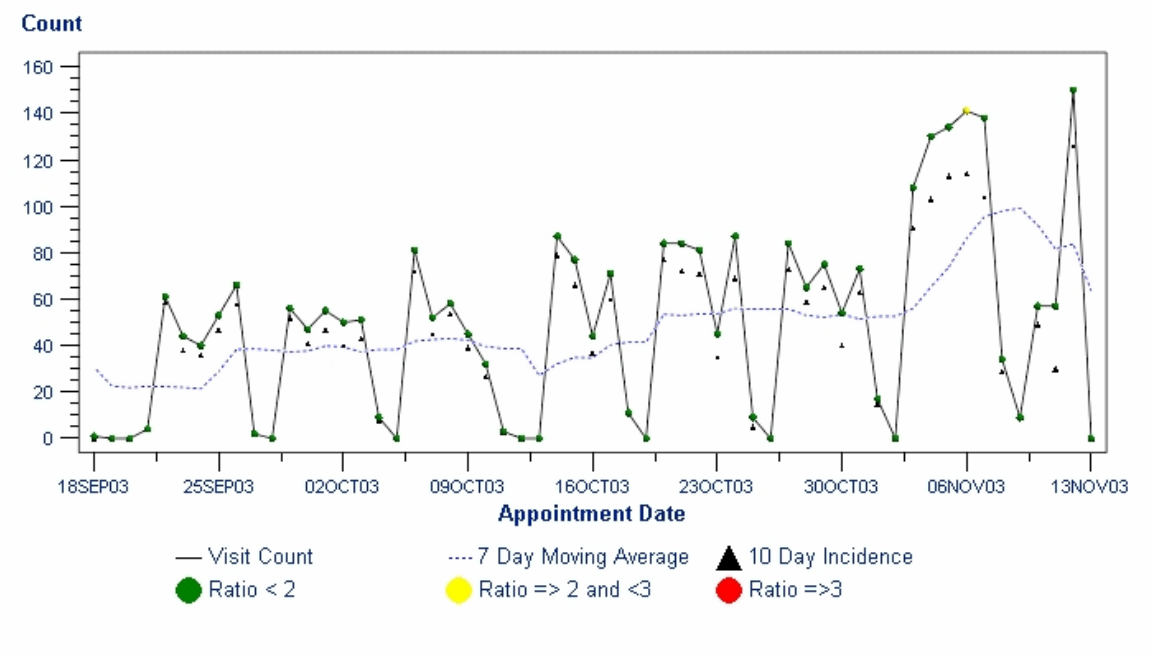

Figure 1: ESSENCE Respiratory Category Graph for Base X

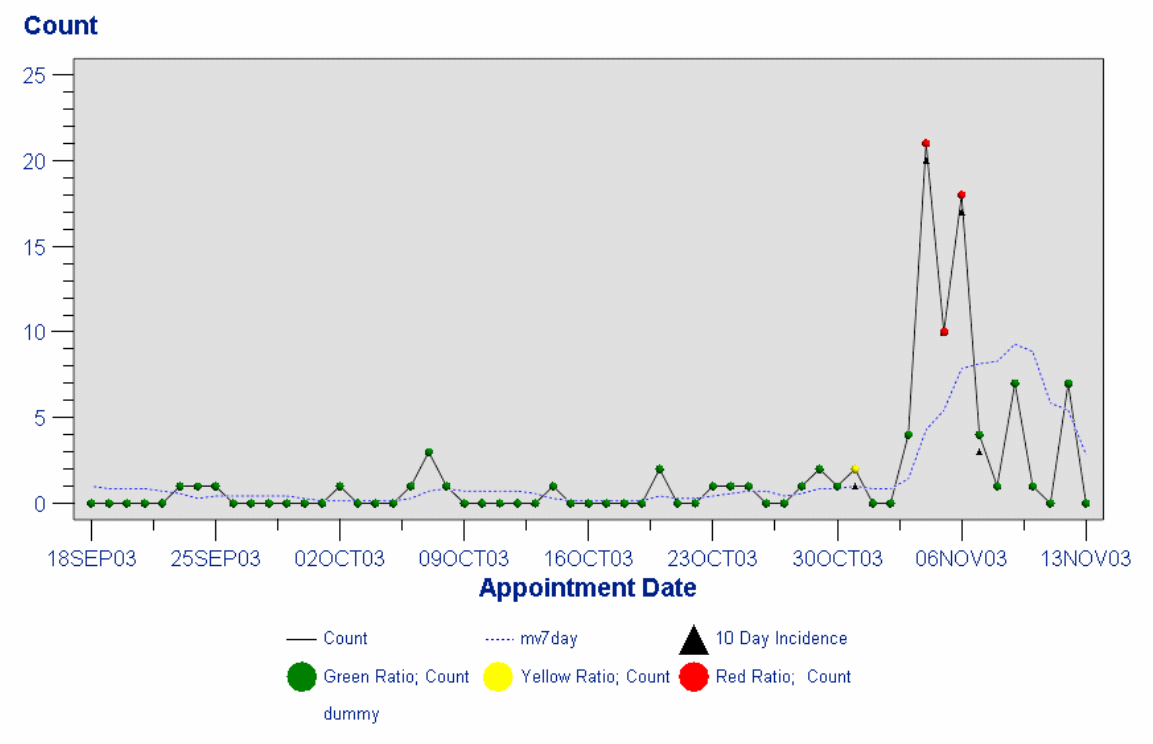

Figure 2: ESSENCE Fever Category Data for Base X

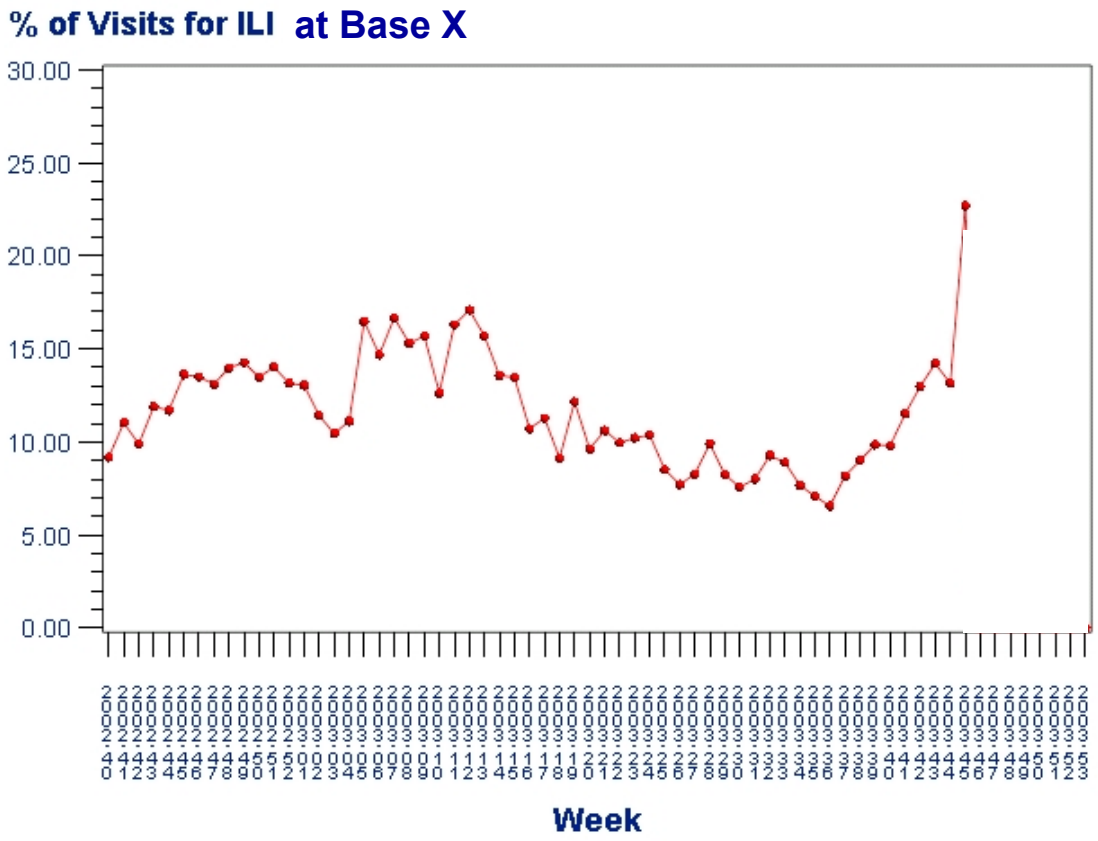

Figure 3: ESSENCE Influenza-like Illness (ILI) Graph for Base X

5. CONCLUSIONS

Emerging infectious diseases and weapons of mass destruction are serious threats to military operations and communities everywhere. Prompt intervention with immunizations, prophylactic medications, or other measures can slow or even stop an outbreak. Such measures are most effective early in the course of the outbreak. Consequently, an active and integrated set of surveillance systems is one of the best ways to stay ahead of biological disease agents. Such integrated and enhanced surveillance systems also may serve as early warning systems for deliberate attacks involving biological agents, though more work is necessary to validate the best types of data and analyses to use for this application.

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[2] http://www.geis.ha.osd.mil/

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[4] Canas LC, Lohman K, Pavlin JA, Endy T, Singh DL, et al. The Department of Defense Laboratory-based, Global Influenza Surveillance Systems. Mil Med Jul;165(7 Suppl 2):52-6 (2000).

[5] https://afioh.brooks.af.mil/pestilence/influenza/

SYMPOSIA DISCUSSION - PAPER 1

Authors Name: Col Cox (US)

Discussor's Name: Dr Reifman (US)

Question:
How does the system compute the threshold to trigger an alert/alarm?

Author's Reply:
It compares the current day's observed count with the mean of the previous six corresponding weekdays. For example, if today is Monday, it compares today's count with the mean of the previous six Mondays.

Yellow Alert = between 2 and 3 standard deviations.

Red alarm = greater than/equal to3 standard deviations.

Authors Name: Col Cox (US)

Discussor's Name: Dr Foster (US)

Question:
Are the web references in paper generally accessible to NATO military staff?

Author's Reply:
Ref #2: public site

Ref #3: not sure, public, I think

Ref #4: restricted to U.S.A government and military

Prevalence and Screening of Mental Health Problems Among U.S. Combat Soldiers Pre- and Post- Deployment

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Relevant Topics: Medical Surveillance, Research epidemiology, psychological support.

The view expressed are those of the authors and do not reflect the official position of the Department of Defense or any of the affiliations listed above.

Classification: Unclassified, approved for public release

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Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

ABSTRACT

INTRODUCTION/ RATIONALE: Mental disorders are some of the most common and disabling medical conditions among military service members. Deployment, particularly to combat zones, has been associated with a variety of mental health, social, and occupational effects, including PTSD (15-40% lifetime rate after combat), depression, substance abuse, job loss, unemployment, divorce, and spouse abuse. To better provide early intervention for mental health problems, the U.S. military has been conducting routine psychological screening since 1996 before and after operational deployments, and has included mental health screening in the post deployment health assessment mandated for troops returning from Afghanistan and Iraq. Despite these efforts, little research has been done to determine the prevalence of mental health problems among combat / operational units, the validity and benefits / risks of screening, or the optimal delivery of mental health services.

METHODS. Data from two major ongoing research efforts will be presented. The first effort includes the results of brief self-administered surveys (including a depression scale, PTSD checklist, and alcohol screening) conducted among nearly 100,000 U.S. soldiers deployed to Bosnia or Kosovo, as well as data from a recent validation study involving 864 soldiers in which all those who screened negative as well as those who screened positive received a brief evaluation by a psychiatric technician. The second effort involves a large study of the impact of deployment and combat on the mental health of U.S. infantry soldiers deployed to Afghanistan and Iraq (N>6,000), utilizing anonymous cross-sectional and longitudinal survey methods (PHQ depression / anxiety instrument).

RESULTS: Of the nearly 100,000 soldiers who received psychological screenings pre- or post-deployment to Kosovo or Bosnia, 15-28% screened positive on the brief self-administered surveys, and 2-12% were recommended for referral. Data from a content validation study indicated that the primary screening methods had high content and construct validity; 37% of the screen positives and 1% of the screen negatives were recommended for referral. In anonymous surveys of soldiers before deployment or within 6 months of return from Afghanistan, 8-10% met screening criteria for depression, anxiety, or PTSD and reported significant functional impairment. There was a low likelihood of using mental health services among soldiers who screened positive; 44% expressed interest in receiving help, but only 24% accessed any services (including chaplains) within the past year. Mental health care was perceived to be highly stigmatizing, particularly by those most in need.

CONCLUSIONS: Approximately 10% of U.S. soldiers in these studies screened positive for a mental health problem and had evidence of functional impairment or need for referral. However, barriers to using mental health services exist, which illustrates the need to reassess how services can be delivered in non-stigmatizing ways. The benefits/ risks of screening will be discussed. The most important potential benefit of screening is the early identification and treatment of mental health problems. Potential risks / costs of routine screening include stigmatization which may have unintentional career effects, and the extensive resources required to conduct screening at the population level. Ongoing research to further validate the instruments and determine the benefit / risk ratio of screening will be presented.

INTRODUCTION

Mental health problems are some of the most common and disabling medical conditions that affect service members (1). Among the 1.4 million active duty U.S. military service members, mental disorders are the leading cause of hospitalization for men and the second leading cause for women (second only to pregnancy-related admissions). Six to ten percent of U.S. military personnel receive outpatient treatment for a

mental disorder each year (1,2). Over 25% of service members who receive outpatient care for mental health problems leave military service within six months, a rate that is more than two times higher than the rate of attrition following treatment for any other ICD-9 illness category (1).

Psychiatric conditions are also important health concerns in operational environments. During Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF), approximately 7% of all evacuations from the operational theater were listed as having a primary psychiatric diagnosis. Many studies have demonstrated the strong link between deployment experiences, especially combat, and a variety of adverse mental health, psychosocial, and occupational effects, including PTSD (15-40% lifetime rate after combat), depression, substance abuse, job loss, unemployment, divorce, and severe spouse abuse (3-6). Available data also indicate that most service members with mental health concerns do not seek treatment, due to stigma and other barriers, although very limited research has been conducted in this area (7,8).

Given the obvious importance of mental disorders among military service members and the unusual stressors experienced during deployment, it would be desirable to have simple and cost effective ways to identify those most at risk. Toward this goal, the U.S. military has been conducting psychological screening before and after many operational deployments, beginning particularly with Bosnia rotations in 1996, in an effort to provide early intervention to those with deployment mental health concerns. In conjunction with the recent operations in Iraq and Afghanistan, the U.S. Department of Defense mandated that all personnel undergo a standardized post-deployment medical screening to include mental health concerns. Despite these efforts, little research has been done to determine the prevalence among service members in combat / operational units, the validity, benefits, and risks of screening programs, and the optimal delivery of mental health services before and after deployment. This paper will review the history of psychological screening pre- and post- deployment in the U.S. military since 1996, the potential risks and benefits of screening, and data from recent efforts to develop validated screening instruments. Current research initiatives will be outlined.

Note that there is much confusion over the term “screening” for psychological or mental health problems, and it is important to define the scope of this paper. The term “screening” is often used to refer to efforts to identify which individuals are fit to join the military and which should be excluded (screening for fitness for duty or recruitment screening). Research has failed to show the effectiveness of this type of screening (9). The term “screening” can also be used to refer to complex selection processes for certain types of assignments, such as aviation, special operations, and law enforcement. This paper will only focus on the medical uses of screening to identify individuals with mental health problems before or after deployment for the purpose of providing adequate services to these individuals so that they can continue to be successful in the military.

PSYCHOLOGICAL SCREENING IN U.S. MILITARY-BOSNIA TO PRESENT.

Deployment psychological screening programs have been used routinely to assess the mental health of soldiers beginning with deployments to Bosnia 1996, in part due to the high rates of stress and physical health concerns observed among veterans of the 1990-91 Gulf War. Initially mandated by the Office of the Secretary of Defense for Health Affairs and developed by the Joint Medical Surveillance Program, the psychological screening included three self administered symptom scales including the Zung depression scale (10), a PTSD checklist that followed DSM-4 criteria (11), and the 4-question CAGE screening test for alcohol abuse (12). As the program evolved, there was some refinement in the instruments including replacing the CAGE, due to very high false positive rates, with the AUDIT (13). Since 1996, over 100,000 soldiers have

been screened using these instruments either just before deployment, during the redeployment process, or just after returning to garrison (14). Rates of screening positive to one or more of these scales has ranged from 15-28% of soldiers. These rates reflect broad screening criteria using generally established cutoff criteria and do not require any evidence of functional impairment or high symptom severity. Those soldiers who screened positive received a brief (~10 minute) clinical screening procedure usually conducted by a psychiatric technician. Of the soldiers who screened positive, 2-12% received a referral for further evaluation or treatment, although it is unknown how many actually followed through with these referrals. Less than 1% of all soldiers screened required immediate referral for an urgent mental health problem.

Data from the program has been used to better define optimal deployment length and the relationship of stress levels and the phase of the deployment cycle (14-16). The program has also potentially served a useful purpose of identifying soldiers in need of additional support from mental health services in the operational setting or after re-deployment home.

Despite these extensive efforts, the deployment screening program has generated questions about the potential risks and drawbacks compared with the benefits. As mentioned earlier, the screening program was originally developed, in part, due to concerns about high rates of mental health and medical symptoms following the 1990-91 Gulf War, but the screening program was not originally designed to clarify the relationship between deployment stressors and post-deployment mental health problems. It is also unknown if psychological screening reduces mental health casualties or improves outcomes among soldiers during or after deployment through early clinical interventions. Questions of the efficacy of early interventions are not limited to screening, but are shared by all initiatives that provide early interventions following exposure to stressors (17).

There are also questions regarding the validity of the instruments used for the purposes of screening healthy adults in operational settings. Although validated in clinical populations, instruments such as the Zung, PTSD checklist, CAGE, and AUDIT have not been validated for use in young, predominantly male healthy soldiers who may be experiencing significant stress related to the deployment. Consistently, rates of screening positive have been lower as soldiers prepare to return to their home station compared with soldiers in garrison or those preparing for deployment (14,16). However, the implications of these differences are uncertain because they are largely based upon cross-sectional data, and do not include combat deployments.

Deployment-related psychological screening is resource intensive, usually drawing mental health professionals out of the traditional clinic setting to administer the screening at the deployment processing points. This may serve a very useful purpose of allowing mental health professionals to interact with soldiers at the unit level, but it is unknown how many of the soldiers with acute mental health problems identified through screening would have been identified through traditional referral routes. Although the screening is designed to provide targeted interventions to those most at risk, the high sensitivity (and corresponding low specificity) results in 15-28% of soldiers needing a face-to-face interview with a psychiatric technician (who themselves require supervision from licensed providers). Thus, deployments involving thousands of soldiers can considerably burden the mental health care system.

In addition, there may be unintended consequences and risks inherent in the screening process. Traditional psychological screening has been associated with stigma during a deployment to Bosnia. (18). In this study, soldiers who screened positive for a psychiatric condition were more likely to feel stigmatized than soldiers who screened positive for another medical condition. During the original mass population screening program, soldiers who screened positive had to stand in a different line to be seen by the psychiatric technician, and other soldiers could easily figure out which line a soldier was standing in. It is unknown

whether this potential stigmatization results in unintended career consequences. Also recent data based on anonymous surveys (8) suggests that perceptions of stigma are higher among soldiers with mental health problems then other soldiers, so it is unknown how the process of conducting the screening may have affected perceptions of stigma. It is also unknown if the high rate of referrals related to this triage system may result in unnecessary treatment and iatrogenic health or adverse occupational consequences for some soldiers. For these reasons, Health Affairs discontinued routine deployment psychological screening after a Combat Stress Control Panel recommended this in 1999.

Despite these concerns, deployment screening programs have continued in the U.S. military for many deployments to Kosovo and Bosnia, and more recently with OIF and OEF rotations. Commanders often encourage psychological screening because they perceive this as a constructive way to take care of soldiers and to show that the organization cares for soldiers. Such programs generate a sense of satisfaction among leaders that something is being done to assure the psychological health of soldiers, particularly in the context of post-gulf war deployment health issues. Soldiers, as well, often report that they appreciate that someone took the time to sit down with them and discuss their stressors. The satisfaction of soldiers and leaders have often drives continued program implementation, despite the fact that these programs have not been assessed as to whether they reduce psychological casualties. One of the interesting findings from the Britt paper was that although soldiers who screened positive for a mental health concern were more likely to feel stigmatized than soldiers who screened positive for another medical issue, they were more likely to view the screening program as beneficial (18). Thus, perhaps stigma is less important to soldiers than receiving help.

Although the data supporting the use of deployment screening is mixed, organizational pressures frequently lead to program implementation, and clinicians assigned to operational units do their best to make these programs a success. Clinicians working with operational units will, of course, not refuse their Commander's request for such a program, where or not there is proven clinical efficacy.

Beginning with OEF/OIF and the large number of troops deployed, DoD mandated an expanded post-deployment health assessment for all returning troops. The assessment consists of a 4 page checklist (Department of Defense Form Number-2796) followed by a brief interview by a primary care clinician who reviews the form and determines if further referral is indicated. Areas covered include location of deployment, environmental and operational exposures, physical symptoms, and mental health. Mental health assessment is limited to questions on potentially traumatic exposures, four questions covering key domains of PTSD, two stem questions for depression, one screening question about suicidal ideation, two questions related to concerns about aggression, and one question about interest in receiving help. The reliability and validity of these questions is unknown.

CURRENT RESEARCH QUESTIONS AND INITIATIVES.

The Walter Reed Army Institute of Research (WRAIR), which includes the Medical Research Unit-Europe, has developed a program to define the extent of deployment-related mental health problems and optimal screening methods. The current research can be broadly grouped into two categories: 1) studies to define the prevalence of mental health problems and determine the impact of deployments among soldiers from operational units and families, and 2) studies to determine the validity, effectiveness, and optimal methods to screen for mental health problems pre- and post-deployment.

PREVALENCE AND DEPLOYMENT RISK-FACTOR STUDIES

Although there is an extensive literature documenting the health, occupational, and social morbidity associated with deployment and combat (3-6, 19-21), most of these studies have been conducted years after combat exposure. Recent studies that have begun to document the burden of mental disorders among U.S. military personnel have been based primarily on health care utilization data (1,2). There have been very few studies that have directly assessed the prevalence of mental health problems among active duty personnel using validated clinical instruments among populations of healthy (non-clinical) service members, especially studies conducted proximal to the time of deployment. Studies have not measured the relationship of deployment and combat to a wide range of outcomes using longitudinal cohort methods among service members surveyed throughout the deployment cycle.

To address these scientific questions, researchers at WRAIR initiated a large study in January 2003 to assess the impact of current military operations in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF)-Afghanistan on the health and wellbeing of soldiers and family members. This study involves both cross-sectional and longitudinal design methods. Soldiers from operational units deploying to OIF and OEF have been surveyed before deployment, and / or after returning from deployment. Post-deployment assessments are being conducted at 3-4 months, 6 months, and 12 months after returning from deployment. Outcomes include depression, anxiety, and PTSD measured by validated self-administered survey instruments. Other outcomes include alcohol use, aggression, and family functioning. Surveys are administered anonymously in an effort to encourage honest answers.

Preliminary analysis of data from soldiers returning from OEF indicates that approximately 10% expressed interest in receiving help for a stress, emotional, alcohol, or family problem, and a similar percent met criteria for depression, anxiety, or PTSD, using a conservative definition that included the requirement that there be evidence of functional impairment or high symptom severity. Among soldiers who screened positive, there was a low likelihood of using mental health services. Only 44% of these soldiers expressed interest in receiving help, and only 24% accessed any services (including chaplains) within the past year. Mental health care was perceived to be highly stigmatizing, particularly by soldiers most in need. Analyses are currently ongoing to determine the prevalence of mental health concerns among soldiers who have returned from OIF.

DEPLOYMENT MENTAL HEALTH SCREENING STUDIES

Beginning in 2001, WRAIR together with USAMRU-E initiated an effort to validate existing screening instruments that had been utilized since 1996, and assess if there were new instruments with higher validity and reliability (14). The primary objective of this program is to develop a simple, easily administered, valid, and cost effective screening procedure for population-level assessment and triage of mental health problems pre- and post- deployment. Evaluation of the new post-deployment health assessment (DD-2796) is also part of this program. In a preliminary validation study, researchers conducted screening among 864 soldiers deploying to Kosovo (rotation 4B - Oct 2002). The primary study objectives were 1) to determine if existing screening criteria are effective in identifying soldiers who need referral (validity), and 2) to determine if the instrument covered the important problems and symptoms experienced by soldiers. In this study, all soldiers who underwent the primary paper screening had a brief clinical interview with a psychiatric technician. Unfortunately, operational constraints made it impossible to keep the results of the primary screen blind from the psychiatric technician who conducted the interview, so it was not possible to directly assess sensitivity and specificity. However, the technicians were able to assess if there was functional impairment,

clarify the responses that soldiers gave on the primary screen, and assess if the content area on the primary screen was sufficient. Of the 864 soldiers who underwent screening, 183 (21%) screened positive on the primary screening instrument, consistent with other deployments.

Of the 183 screen positives, 68 (37%) were recommended for referral (5 immediate) and 67 (37%) had "sub-clinical" complaints that did not require referral. Less than 1% were assessed as non-deployable. Of the 681 screen negatives, only 7 (1%) were identified as having problems sufficient to recommend referral (all had relationship problems). No new content areas were identified in the interviews that were not already included on the screening instrument. Study findings also indicated that the existing screening tools had construct validity. Positive screening was associated with impaired social / occupational functioning, past history of counseling or childhood adversity (trauma), and family history of mental health problems. The study represented the first step at assessing validity of the screening.

Data from the most definitive study on the validity of the deployment psychological screening program has just been collected among 1,600 soldiers returning from a one-year deployment to Iraq. In this study personnel who conducted the secondary clinical interviews were kept blind to the results of the primary screen and they used a standardized structured diagnostic interview following DSM-IV criteria for PTSD, depression, and alcohol abuse using the MINI (22). The secondary interview was conducted among all soldiers who screened positive on the primary screen and a random sample of 20% of the soldiers who screened negative. The new procedure of interviewing soldiers who screened negative not only allows for validity checks, but also serves the purpose of reducing stigma. Soldiers were briefed in advance abut the random nature of the secondary interview. Analyses of these data are ongoing. In addition to validating the instruments used in the Kosovo and Bosnia deployments, this study will also provide data on the optimal primary screening instrument, to include the PHQ for depression (23) and the questions included on the post-deployment health assessment.

CONCLUSION.

In summary, mental disorders are an important cause of morbidity among military service members, and it likely that there will be significant mental health effects of recent operations in Iraq and Afghanistan. Developing validated deployment screening programs linked to prevention and early interventions is a high priority. The most important potential benefit of deployment screening is the early identification and intervention of mental health and behavioral problems, which in turn may reduce the chronicity or severity of these conditions, improve soldier and family functioning, and improve unit readiness. In addition, screening serves the purpose of making mental health services immediately available to soldiers thereby reducing one of the key barriers to care. In any pre-deployment screening program there are always a few soldiers who are identified who should not deploy due to overt mental health problems (e.g. severe symptoms, such as withdrawal from alcohol dependence). This accounts for less than 1% of soldiers who are screened, and it is unknown how many of these soldiers would have been detected through usual command directed referral routes. For post-deployment screening, there is the potential to identify soldiers who are at high risk health concerns and facilitate appropriate referral. Other potential benefits include the Commanders' and soldiers' satisfaction that something is being done to address the psychological effects of deployment. Commanders often request screening, and soldiers often report appreciation that the military is taking an interest in their well-being. The argument that screening provides a way to target resources to a segment of the population that is most in need may be offset by the resource intensive nature of the screening program itself.

Potential risks and drawbacks of routine screening include stigmatization with unintended career consequences. Further work is needed to devise procedures to reduce stigma. In addition, screening may result in potential iatrogenic effects of referring large numbers of persons to mental health. Soldiers may inadvertently identify themselves as a patient because they have been positively screened. There is also a lack of data showing that screening methods have reasonable sensitivity, specificity, and predictive value in correctly identifying those who would benefit from referral.

Ongoing research includes an extensive study of the impact of current operations in Iraq and Afghanistan on soldiers and families and the first direct validation of deployment mental health screening using structured diagnostic interviews conducted by persons who are blind to the results of the primary screen. Further research is needed to develop optimal procedures for survey administration. There are important distinctions between deployment screening programs designed to identify individuals who may be at risk, and anonymous needs assessments at the population level designed to measure the behavioral health needs in the population. Anonymous surveys also may encourage self-referral. Defining the appropriate context and purpose of the screening program, and developing a better understanding of the risks and benefits of different screening procedures is critical. By prioritizing both early identification and epidemiological surveillance, the U.S. military is establishing a basis from which to develop optimal screening programs.

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SYMPOSIA DISCUSSION - PAPER 2

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Discussor's Name: Dr Lam (US)

Question:
Pre-deployment screens are mostly at time of high stress – Would it not be more useful to screen garrison communities rather than immediately pre-deployment?

Author's Reply:
Yes, some studies are being done in this environment.

Air Pollution and Prevalence of Allergic Diseases in Georgian Adolescent Population

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SUMMARY

During the last decades special attention of scientists has been focused on environmental pollution intensity and a great number of the so-called outdoor allergens (SO_2, NO_2, phenol, combustion gases, etc.) as well as on their increasing effect of formation of allergic diseases. Children's population is most sensitive to the influence of unfavorable environmental factors. This is associated with a number of physiological peculiarities of a children's organism.

In the present work the relationship between the frequency of allergic diseases in young population and the air pollution intensity has been studied. With this purpose in Tbilisi (Georgia) National Environmental Monitoring Center data on the laboratory control of air samples from seven districts of Tbilisi have been collected.

The prevalence and structure of allergic diseases (bronchial asthma, polynosis, atopic dermatitis, urticaria and Quincke's edema) have been investigated by examining an occasional representative group of adolescents (11073 adolescents aged 12-19 years) according to specially developed unified methods including a screening-questionnaire, a detailed map of epidemiological-biological analysis and unitary diagnostic criteria of allergic diseases in children.

The analysis of the data obtained has shown the relationship between the increasing prevalence rate of allergic diseases in children and the chemical air pollution intensity ($p<0,001$). In the districts with high

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air pollution intensity the earlier onset, heavier development of allergic diseases in children and the increase of the polyvalent sensitization frequency ($p<0,05$) have been recorded.

Thus, the obtained results indicate a significant influence of the air composition on the prevalence of allergic diseases and its growth with the pollution intensity in the dwelling district.

Problem Statement

Georgian Armed Forces is been forming now. One of the most important problem of nowadays military medicine very high morbidity of military personnel. Despite the fact that the scale and quality of conscripts' medical investigation, that is being done at the moment of recruiting, is far from required standards, - above mentioned morbidity level is a little bit unexpected.

We have done randomized investigation of patients' histories in Central Military Hospital (Tbilisi) and Military Outpatient Clinic (Tbilisi) for the last 5 years period: 1998-2003. Among the frequently revealed diseases, are allergic disorders: urticaria, Quinke's oedema, chronicle rhinitis, conjunctivitis and bronchial asthma. Above mentioned diseases often became the causes of soldiers' retention from the army. Their frequency are as well high in the civil population of similar age group.

In conditions of a substantial increase in bronchial asthma incidence rate epidemiological investigations become very important. It should be mentioned that none of the official documents of WHO and European international societies gives information on epidemiological investigations carried out in the former USSR. This can be explained, in official data such as low level of the bronchial asthma incidence rate is given that this figures are regarded as rather doubtful. In order to obtain impartial information on bronchial asthma in adolescent and young population in Tbilisi a prospective epidemiological investigation has been carried out.

During the last decades special attention of scientists has been focused on environmental pollution intensity and a great number of the so-called outdoor allergens (SO2, NO2, phenol, combustion gases, etc.) as well as on their increasing effect of formation of allergic diseases (1,2). Adolescents' population is most sensitive to the influence of unfavorable environmental factors. This is associated with a number of physiological peculiarities of a children's organism (3,4).

For this viewpoint the study of the role of ecological situation in formation of allergic diseases in Tbilisi becomes very important. The economical and geographical location of Tbilisi and expansion of urban building leads to widening of external and internal urban communications network and decreases the traffic capacity. High motor transport intensity and low traffic capacity increase the increase amount of combustion gases and exert the negative influence on the ecological situation in the city . Intensive urbanization processes all over the world will continue to affect adversely on prevalence of allergic diseases even if all city industrial enterprises are closed down. Motor Transport and fuel combustion are inevitable associated with an exhaust of a carbonic acid gas, carbon monoxide, nitrogen oxides, polycyclic hydrocarbons and other allergy inducers. It has been established that xenobiotics absorption on pollen and other allergens increases their immunogenicity a hundred times, most active are formaldehyde, thyocianates, nitrogen, and sulfur oxides and toxic radicals (5, 6, 7).

Materials and methods

In the present work the relationship between the frequency of allergic diseases in children and the air pollution intensity has been studied. With this purpose in Tbilissi National Environmental Monitoring

Centre data on the laboratory control of air samples from seven air pollution observation zones in seven districts of Tbilissi have been collected.

The epidemiological investigation was performed by stages:

1. First stage – initial screening-questionnaire according to the specially developed program. Using the extended questionnaire (240 questions) a seasonal character , course of the disease, reasons for onset and recurrence, premorbid background, associated diseases duration of the disease etc. were revealed. At this stage 178 bronchial asthma patients were selected and then examined in special day hospital;

2. Second stage – questioning of the subjects selected at the first stage was carried out according to the extended map of epidemiological investigation;

3. Third stage – examination of some patients using special diagnostic methods: such as skin tests, determination of a general IgE level and specific Ige antibodies in blood serum by the ELISA method, investigation of the external respiratory functions; general medical examination methods were also used.

The epidemiological investigation data were processed using the program package SPSS.

The prevalence and structure of allergic diseases (bronchial asthma, polynosis, atopic dermatitis, urticaria and Quinke's oedema) have been investigated by examining an occasional representative group of adolescents (----- children aged year) according to specially developed unified methods including a screening-questionnaire, a detailed map of epidemiological analysis.

11073 adolescent and young population of 12 to 20 years of age living in Tbilisi were investigated. Among theme were 200 cadets from United Military Academy aged 15-18 years and 200 military men aged 18-20 years.

The difference of allergic diseases incidence rates between population depend not only on different approaches of its revealing, but also on different exposition factors which can provoke these diseases. In this connection in the work the frequency of possible combination of medical and biological as well as social and hygienic risk factors and their qualitative distribution in young population. were studied.

Results:

In Tbilisi the prevalence of allergic diseases in children was 18.8%. Atopic dermatitis has the highest incidence rate – 7.7%, urticaria and Qunke's oedema are recorded in 4.4 % of cases, polynosis amounts to 3.1% of cases.

The initial screening questionnaire of the representative group of population by yard-to-yard round revealed 343 children with bronchial asthma , which forms 3.1. Among them 147 are female, 196 – males.

Bronchial asthma was prevalent in the groups of 12-15 years old adolescent and more rarely was observed in the older group.

More often genetic burden was observed from maternal (52.8) and more rarely from fraternal (21.6) line. Inheritance was burdened by allergic diseases both from maternal and fraternal line (21.6). Parents (and close relatives) of these patients suffered from various allergic diseases.

Among patients from bronchial asthma the presence of one risk factor was observed only in 2% of cases and in the rest cases – a combination of several (3,4,5) factors was noted.

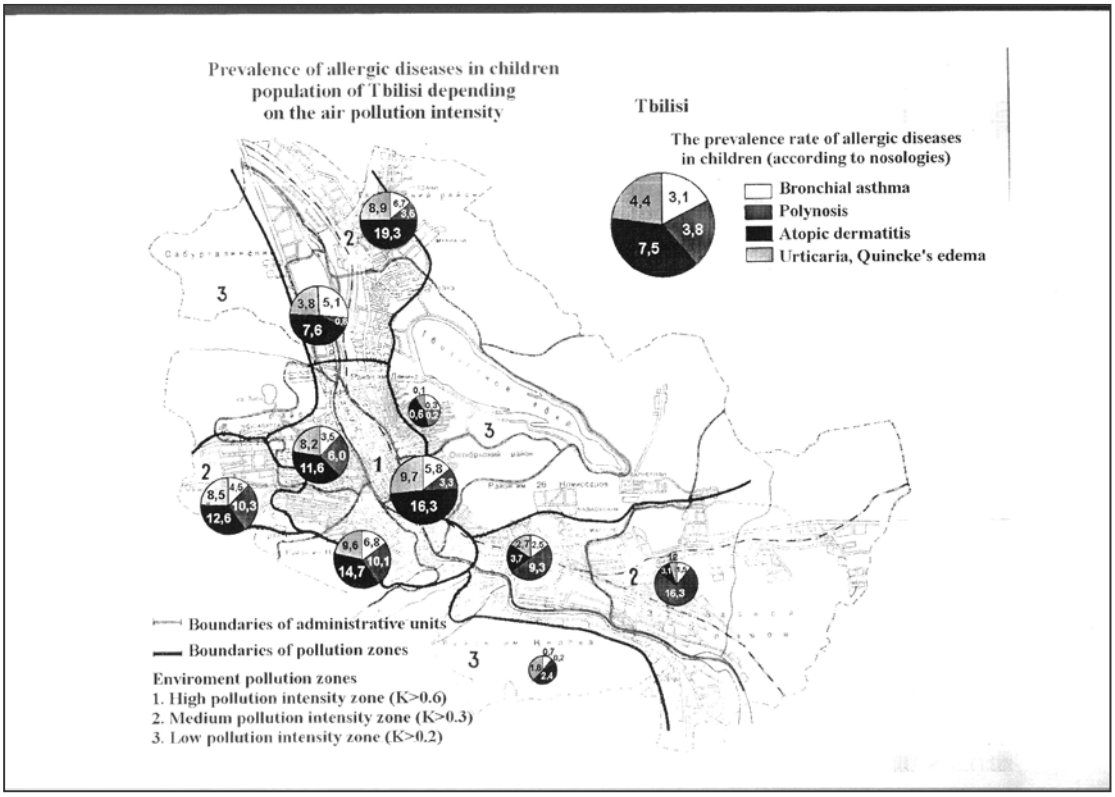
At the third stage selected patients were examined in special day hospital. In 101 case atopic bronchial asthma was revealed, and in 77 cases – infection-depended asthma was noted. In all the cases of atopic bronchial asthma an increase in the general IgE level was observed, but at the infectious-depended asthma cases the general IgE level was normal. Disorders in the external respiratory functions were more significant in at the infectious-depended asthma.

In 78 cases of atopic BA the aetiologic structure was established by determining specific IgE antibodies in blood serum. In 23 cases Skin tests were performed. The casual factor of infectious-allergic asthma (77 cases) was also revealed by skin tests.

The Investigation of bronchial asthma in young population of Tbilisi showed an intensive increase in bronchial asthma incidence rate for the last two decades, namely by a factor 14. Sex dependence of the disease was noted and age groups susceptible to bronchial asthma were distinguished. Some regularities in the study of the risk factor structure were found. Frequency and peculiarities of distribution of separate factors and variants of their possible combination in bronchial asthma and healthy population were established. Casual factors of BA seemed to be first of all food and social allergens and then pollen, epidermal and bacterial sensibilization.

The analysis of the air pollution intensity in the studied districts has shown a different quantitative and qualitative air composition. See map# 1 .The composition of the air pollution data with maximum allowable concentrations (MAL) in separate chemical components revealed different Contamination levels in the investigated zones. In the first MAC was exceeded in 3 components (dust – by a factor of 3.8; carbon monoxide – by a factor of 1.5, phenol – by a factor of 2, formaldehyde – by a factor 4.2). In the second zone MAC was exceeded in 3 substances (dust – by a factor of 4.3; phenol – by a factor of 1.6, formaldehyde – by a factor 5.6). In the third zone the excess of MAL was observed in 2 components (dust – by a factor of 4.3; phenol – by a factor of 1.6, formaldehyde – by a factor 5.4). In the fourth zone MAL was exceeded of in 4 components (dust – by a factor of 1.6, carbon monoxide – by a factor of 1.2, phenol – by a factor of 2, formaldehyde – by a factor of 5.4). In the fifth zone – in two components: (dust – by a factor of 2.4, formaldehyde – by a factor 4.2), In the sixth zone – in three components: dust – by a factor of 2.4, carbon monoxide – by a factor of 1.4, phenol – by a factor of 1.7) and in the seventh zone no mark able excess of MAC in two substances was found (dust – by a factor of 1.4; phenol – by a factor 1.2). Thus in all the investigated zones the excess of substances of the second and third class of danger was revealed.

Picture 1



Taking into account the different composition of air pollutants in the districts under investigation the net air pollution index (T) (see table #1), was used for a comparative hygienic assessment of the pollution intensity. This allowed one to classify the investigated districts in conventionally pure (Nadzaladevi). With relatively low air pollution intensity (Samgori and Issani) and polluted (Chugureti, Gldani, Saburtalo and Didube) districts.

Table 1: Relationship Between the Prevalence Rate of Allergic Diseases in Young Population and the Air Pollution Intensity

District	Number of examined patients		Prevalence of allergic disease		Bronchial Asthma		Polynosis		Atopic dermatitis		Urticaria and Quinke's edema		Net index of air pollution (T)
	abs.	%	abs	%	abs	%	abs.	%	abs.	%	abs.	%	
Nadzaladevi*	2354	21.3	30	1.3	8	0.3	6	0.2	13	0.6	3	0.1	3.94
Vake	667	6	255	38.3	30	4.5	69	10.3	83	12.6	72	8.5	
Chugureti*	1371	12.4	492	35.1	80	5.8	46	3.3	223	16.3	132	9.7	16.3
Krtsanisi	1849	16.7	92	5	12	0.7	3	0.2	44	2.4	33	1.8	
Samgori**	596	4.9	121	22	8	1.5	89	16.3	17	3.1	7	1.2	6.63
Isani**	733	6.6	133	18.2	19	2.5	69	9.3	27	3.7	14	2.7	7.72
Saburtalo***	951	8.7	278	29.2	33	3.5	57	6.0	110	11.6	78	8.2	10.78
Mtatsminda	521	4.7	215	41.2	36	6.8	53	10.1	76	14.7	50	9.6	
Didube***	1451	13.1	253	17.3	75	5.1	11	0.8	111	7.6	54	3.8	10.29
Gldani***	631	5.7	242	38.4	42	6.7	22	3.5	122	19.3	61	8.9	14.55
Total	11073	100.0	2035	18.8	343	3.1	425	3.8	828	7.5	489	4.4	

The analysis of the data obtained has shown the relationship between the increasing prevalence rate of allergic diseases in adolescents and the chemical air pollution intensity ($p<0.001$). So, in the pure district it was 1.3%. In the contaminated zones the allergic disease prevalence was high: 7.3% - 38.4%.

In the districts with high air pollution intensity the earlier onset, heavier development of allergic diseases in adolescent and young population and the increase of the polyvalent sensitization frequency ($p<0.05$) have been recorded.

Thus, the obtained results indicate a significant influence of the air composition on the prevalence of allergic diseases and its growth with the pollution intensity in the dwelling districts.

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Suicide among Veterans: Research, Models and Data

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ABSTRACT

Research among Vietnam veterans shows that PTSD and feelings of shame and guilt are risk factors for suicide. Many taboos are covering the subject of suicide. Data on suicide of Canadian peacekeepers seem to show that these peacekeepers are not at risk for suicide. However, Norwegian peacekeeping veterans are at risk for suicide as well as some risk groups of active duty military personnel. Models for explanation and prevention of suicide can identifyrisk factors and protective factors for suicide. Research and data on suicide have strong implications for military mental health care.

1.0 Introduction

Since 1990 80.000 of Netherlands military personnel have participated in peace-keeping and peace-enforcing operations of the United Nations and NATO all over the world. In 2001 a Canadian study on suicide among Canadian veterans was published (Wong et al, 2001), in which study the question was answered whether Canadian veterans are more likely to commit suicide than other people of their age. According to the Canadian authors, the answer is negative. However, in our meta-analysis we will comment on this study and we will try to answer this question in a different way (Meijer and Weerts, 2002). First of all, we will examine some studies on suicide among Vietnam-veterans. Then we will analyse the Canadian study. From these studies we will derive a model with factors that can explain why people commit suicide. Combined with three cases of suicide among Netherlands military personnel, we will also introduce a model that can be used for prevention of suicide. From this last model and other data on suicide amon active duty military personnel we will derive some consequences for policy on care for military personnel as well as for veterans, military personnel that has been participating in operations and has ended active service.

1.1 Taboos on suicide

We have to remark that at least two strong taboos rest on the subject of suicide. The first taboo comes from death itself. From research on care for patients in hospitals it appeared that patients who were

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terminally ill, got fewer visits and had to wait longer for help, once they called for that help (Van der Meer, 1970, p.19). This author cites that in an American conference about care in psychiatric hospitals a conference-member shouted 'we do not even permit the dying person to say goodbye to us' (Van der Meer, 1970, p.20). The second taboo rests on suicide, the subject that is denied in every way out of so-called respect, shame or guilt (Bach, 1992, p.24). It is possible that this so-called respect comes from the belief that keeping this subject away from public awareness might decrease the risk of suicide, because you haven't heard or haven't thought of it. This very weak justification of the taboo is that it prevents suicide. In our opinion this prevention-effect of the taboo of not thinking, talking or studying suicide is a strong example of wishful thinking or denial. Especially shame and guilt are connected with the biblical curse on suicide, although in the bible there are many people who commit suicide and even the well-known Dutch theologist Kuitert states: 'suicide is a disaster, not a crime´ (Bach, 1992, p. 30). Maybe a specific taboo on suicide among veterans may rise from the responsibility of the Department of Defence for the well being of their personnel and the chance of societal or individual claims, once it appears that the incidence of suicide among military personnel or veterans is high. Even the strongly felt individual responsibility for taking care of suicidal people, once it is too late, can foster this taboo. However, we conclude that only revelation of the taboo and close examination of the subject can lead to a better understanding of suicidal behaviour and the possibilities of prevention. Therefore, this paper aims to bring the subject out into the open.

2.0 Research on suicide among veterans

In 1975 the United States of America ended its war in Vietnam. More than 3 million American soldiers served in Vietnam for a tour of duty of one year most of the time (Shephard, 2001, p. 340). Of these soldiers approximately 57.000 were killed in action, a much larger number was wounded in action and an unknown number is still missing in action. Many had stayed physically impaired, due to their wounds. In the years after 1975 a still unknown number committed suicide, or was killed in violent deaths.

Box1

The aftermath of Vietnam first really claimed public attention on the 30th of April 1970, the day that Sergeant Johnson was shot.

In Vietnam, Sergeant Dwight Johnson had won the Medal of Honour, the United States' highest decoration for valour, for single-handed knocking out twenty enemy soldiers during a raid on his position. He had then served with distinction for another two years, but on returning home, found difficulty in readjusting to civilian life. He became convinced that the Army exploited all black soldiers and made no effort to help them afterwards: Army psychiatrists did not change this view. His frustration grew until he decided to deploy in his rundown Chicago neighbourhood skills he had shown in Vietnam. He was robbing a liquor store when he was killed...(Shephard, 2001, p.357).

Studies on the number of suicides raised several questions, because the results are quite contradictory to each other. In chronological order we will discuss several studies on the subject of suicide among veterans. We will also make some critical remarks on every study.

Pollock et al. (1990) describe how in United States' mass media repeatedly published numbers of more than 50.000 suicides among Vietnam veterans. This number almost equals the number of soldiers killed in action. Their research upon a sample of Vietnam-veterans leads to the conclusion that extrapolation of the

number of suicides reveals a number of suicides in the total population fewer than 9.000. This study dramatically shows how little data have been kept systematically on the incidence of suicide among veterans. In spite of all care for veterans this appears to be a weak area. Although the absolute estimated number of suicides is relatively small, they also conclude that Vietnam veterans are 25% more vulnerable for suicide than their peers of age and gender. In the end they contrast some remarkable findings on suicide. Most of the time, a depression precedes a suicide and, in general, these depressions occur among females two or three times as often as among males. However, the incidence of suicide among males is three times the incidence of suicides among females. We assume that these paradoxical findings can be explained when we take into account that the data on depressions are collected by questionnaires. From the female sex-stereotype, especially expressiveness, it can be expected that females express their depressed feelings in these questionnaires more accurately than males. The male sex-stereotype of competency can explain why so many attempts in suicide are successful, although this explanation is a rather cynical one.

Hendin and Pollinger-Haas (1991) conclude in their research on suicide among Vietnam veterans that especially feelings of guilt, coming from surviving beloved comrades as well as from killing defenceless people like prisoners of war, elderly people, women and children, are the main reason for suicide. Killing out of fear or rage has the most distinct relation with suicide. Attacking enemy villages by order, in which also civilians were killed, is less strongly related with guilt and suicide. They also found out that Vietnam veterans are between 11 to 65 % more likely to commit suicide than non-veterans. Their conclusion that feelings of guilt are the most powerful predictors of suicide resemble findings from research among veterans of the Second World War. The strong benefit of this study is that feelings of guilt appear to be an intermediating factor between having killed people and suicide: these feelings were not identified as such in former research. Especially in psychotherapy for veterans these feelings of guilt have to be worked through, in spite of shame and hesitation of mentioning them. In our opinion this shame and the importance of guilt need to be addressed, even though they are also likely to be a taboo.

Kramer et al (1992) include into the subject of suicide also life-threatening behaviours, like motor accidents, shootings alike the example in box 1 and overdoses of alcohol and drugs. They also include thoughts of death and dying into their research. Figure 1 shows their findings on thoughts on death and dying, their own death and thoughts of suicide among veterans in general, an outreach group of veterans and veterans in therapy.

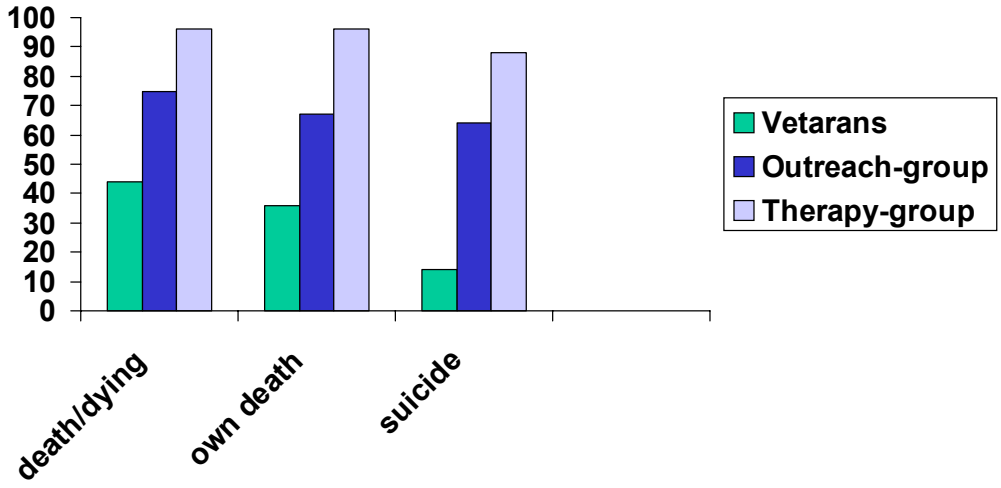

Figure 1. Thoughts on death and dying, the own death and thoughts on suicide among veterans, an outreach group of veterans and veterans in therapy (Kramer et al. 1992).

From figure 1, it appears that veterans in therapy think most of death and dying, one´s own death and suicide. Remarkably, the outreach-group has the strongest psycho-social problems, like unemployment and divorces, as shown in figure 2.

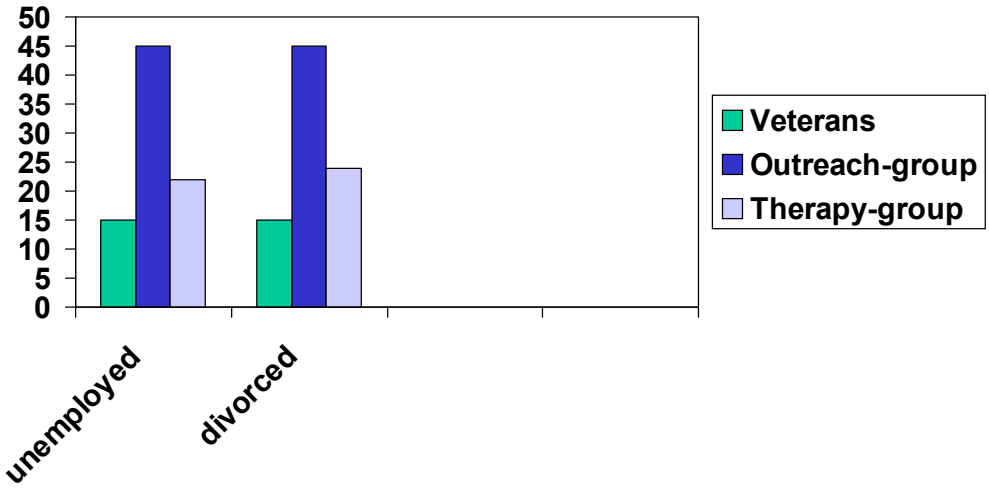

Figure 2. Percentages of people unemployed or divorced among veterans, an outreach group of veterans, and veterans in therapy (Kramer et al. 1992).

The authors do not offer an explanation for the differences between psychological and psycho-social problems among these groups. Probably, the fact that in therapy there is much focus on trauma, events in which death always plays an important role, can explain why the in group in therapy reports more thoughts on death, dying and suicide. The outreach-group can have more severe psycho-social problems, because in many cases partners or colleagues of veterans stimulate veterans to go in therapy. Once these partners have been lost by divorce or job-loss, veterans will not reach therapy. At the end of the day, both figures demonstrate very clearly that veterans in therapy and the outreach group have severe psychological and psycho-social problems.

Bullman and Kang (1994) also include in their study on suicide among veterans violent death by motor-accidents, shootings and overdoses. Further on they connect the PTSD-symptoms of avoidance and numbing with anomy, the utter lack of values and norms as described by the famous sociologist Emile Durkheim. Durkheim (1960) has introduced the concept of anomy to explain why people commit suicide. 'Inflation of values causes anomy and anomy causes suicide' is his conclusion. The authors notice that among Vietnam veterans, especially the veterans who have worked with Agent Orange, a very poisonous defoliant, are up to 4 or 6 times more likely to commit suicide than veterans who have not worked with Agent Orange. Within the group of Agent Orange veterans, a diagnosis of PTSD increased the chance of committing suicide with 71 %. There is a very accurate registration of Agent Orange veterans. The authors give no explanation for the differences in chance on suicide they found. Probably the feelings of guilt as described in the study of Hendin and Pollinger-Haas, coming forward from spreading the poison on defenceless people, including elderly people, women and children, can explain the increase in suicide among Agent Orange veterans. Also the fact that most of these veterans come from the Air Force, that brings personnel to work together for only very short times due to a frequent and individual rotation scheme, can explain these differences, as will be discussed later on in this paper.

Fontana and Rosenheck (1996) conclude from their research among 1000 veterans of the National Vietnam Readjustment Study (Kulka, 1990) that suicide among these veterans remains largely

unexplained, in spite of their research. However, they have not taken into account feelings of guilt, which appeared to be of major interest in explaining suicide. Important veterans-issues, like the homecoming of these veterans and their societal recognition have been remarkably well operationalised in this study. Lack of help in homecoming and lack of possibilities to talk about Vietnam correlate with Post Traumatic Stress Disorder (PTSD) .40 and .30 respectively. Depression and PTSD correlate with suicide between .20 and .34, which means that only a little more than 10 percent of the variance is being explained by these factors. However, the methods used in their data-analysis leave much room for discussion and we already mentioned that issues like feelings of guilt could explain much more about suicide.

Wang et al. (1996) describe the cyclical process of PTSD. In their model they connect with the stages of Horowitz, emotional outcry, denial, oscillation between reacting and numbing, acceptance and solution (Horowitz, 1978). In these stages, the oscillation between reacting and numbing seems to be most beneficial for reaching the final stage of solution (Epstein, 1989). Meijer and De Vries (2001) concluded on their help to veterans that especially very opposite reactions on very different aspects of a situation have to be discovered and worked through. For instance veterans who survived an air-raid in their Mitchell B-25 during the Second World War, do have to experience the relieve of survival, next to the fear of almost being killed and the grieve of losing so many comrades, who have been less lucky. Figure 3 shows this oscillating between aspects of events and the reactions of veterans, divided into observations, behaviour and feelings.

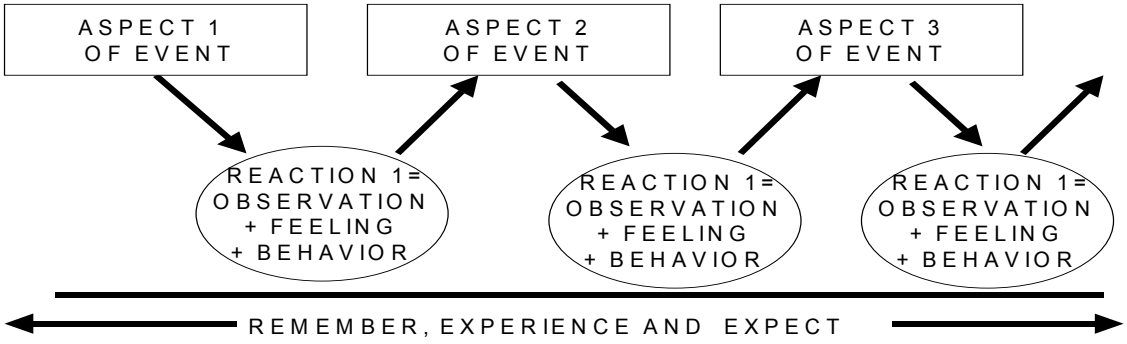

Figure 3. Oscillation between aspects of events and reactions, which consist of observations, feelings and behaviours.

Wang et al found out that in more long-term life-stages, adaptation to demands of every day life can be followed by a stage of surviving, in which general functioning is being impaired. In crossing the threshold to decompensation, veterans loose their jobs and or their relation with a partner because of divorce by behaviour that varies between utmost excitement (runs) and total isolation (bunker), in which every perspective of the future is being lost. The next and last stage is regrouping or getting lost. Veterans regard this last stage to be very similar to the stage after the battle in a combat zone, in which comrades are being found or lost. In addition to the immense feelings of relieve and grieve from their past experiences, also the run and bunker behaviours add strong feelings of guilt and shame from their present experiences. In that stage, for many veterans death by suicide or violent behaviour seems the only way out. We strongly agree with the authors that this cyclical character needs attention in both therapy and research. The authors also note that medication only offers a temporary solution and that the majority of Vietnam veterans has strong relational problems and up to 70 % of them has been divorced (Kulka et al, 1990). Therefore the authors invite researchers to do more research on long-term effects of PTSD.

Wong et al. (2001) conclude on their research among Canadian veterans of peacekeeping operations of the United Nations that these veterans are not more likely to commit suicide than other Canadians of the same age. In our opinion they ignore the fact that military personnel is selected upon physical and psychological health. Therefore their research hypothesis should predict less suicide among military personnel than among civilians. Upon this hypothesis their results support the conclusion that participation in peacekeeping operations increases the likeliness of suicide. It seems that in this study this 'healthy worker effect' has been ignored. They do conclude that the likeliness of suicide increases among air force personnel that have been participating in peacekeeping operations. As noted in the research upon air force personnel of the Agent Orange Administration this might be explained by the short time that air force personnel work together. This personnel is being rotated very frequently and often on an individual rotation-schedule. Further on there are indications that some of the Canadian military personnel participate in peacekeeping operations as often as possible, because of the rewards and bonuses upon their normal wages, that can be interpreted as risk-taking behaviour. These indications need more careful investigation.

Another source of evidence for higher risk on suicide and even cases of suicide comes from military personnel, when they are still acting in peacekeeping operations. Especially military personnel from third world countries seem to be vulnerable to this kind of suicide. In considering their situation we have to realise that the norm of 'rich helping poor', does not apply in their situation, especially when their wages are withhold from these soldiers, which seems to be the case quite often. Also worries about their own families, who are facing the same or even worse problems than the civilians they are supposed to help in their peacekeeping operations, can contribute to the anomy, as discussed before. It might be possible to consider these military personnel as displaced persons in more perspectives. These indications need more careful investigation as well.

Upon the studies we have discussed so far, we conclude that depression, PTSD and feelings of guilt are strong predictors of suicide. Feelings of guilt come from surviving, when beloved comrades have been killed in action or accidents, but also from killing defenceless people, prisoners of war, elderly people, women and children. Participation in peacekeeping operations, which can cause strong feelings of powerlessness and frustration, stemming from watching warfare or atrocities without the possibility of intervening and ending them, can also increase the likeliness of suicide. This possibility needs further investigation as well, but cannot be discussed in this paper. We will continue this paper with a model that summarises factors that explain suicide, and a model for prevention of suicide. Next, we will present and discuss data on suicide among military populations and implications of our analyses for military mental health care.

3.0 Models for explanation and prevention of suicide

All studies discussed before have in common that they try to predict suicide from depression and PTSD, which are strongly interrelated. Only one study adds feelings of guilt as another important predictor of suicide. We will connect these predictors with societal, social and individual factors and world-esteem and self-esteem as hidden constructs that connect these factors and predictors. World-esteem refers to the beliefs that the world is a right place to be, where rich care for poor, strong care for weak and goodness is being rewarded and badness is being punished. Self-esteem refers to the beliefs that the person himself is attractive, strong, honest and healthy. Feelings of guilt affect both world-esteem and self-esteem, and are being added to our model for bringing in these important feelings, as predictors of suicide, hidden in these constructs. We choose to do so because of the fact that almost every traumatic experience affects world-esteem and self-esteem. By experiencing trauma, people realise that the world is not a safe place to be, and that the own personality is not as powerful or attractive as one thought. In working through trauma, a rebuilding of world-esteem or self-esteem is possible, but in this paper we will focus on what happens without this working through. Based upon these predictors of suicide, societal, social and individual

factors, world-esteem and self-esteem that connect them, in figure 4 we present our model to explain suicide.

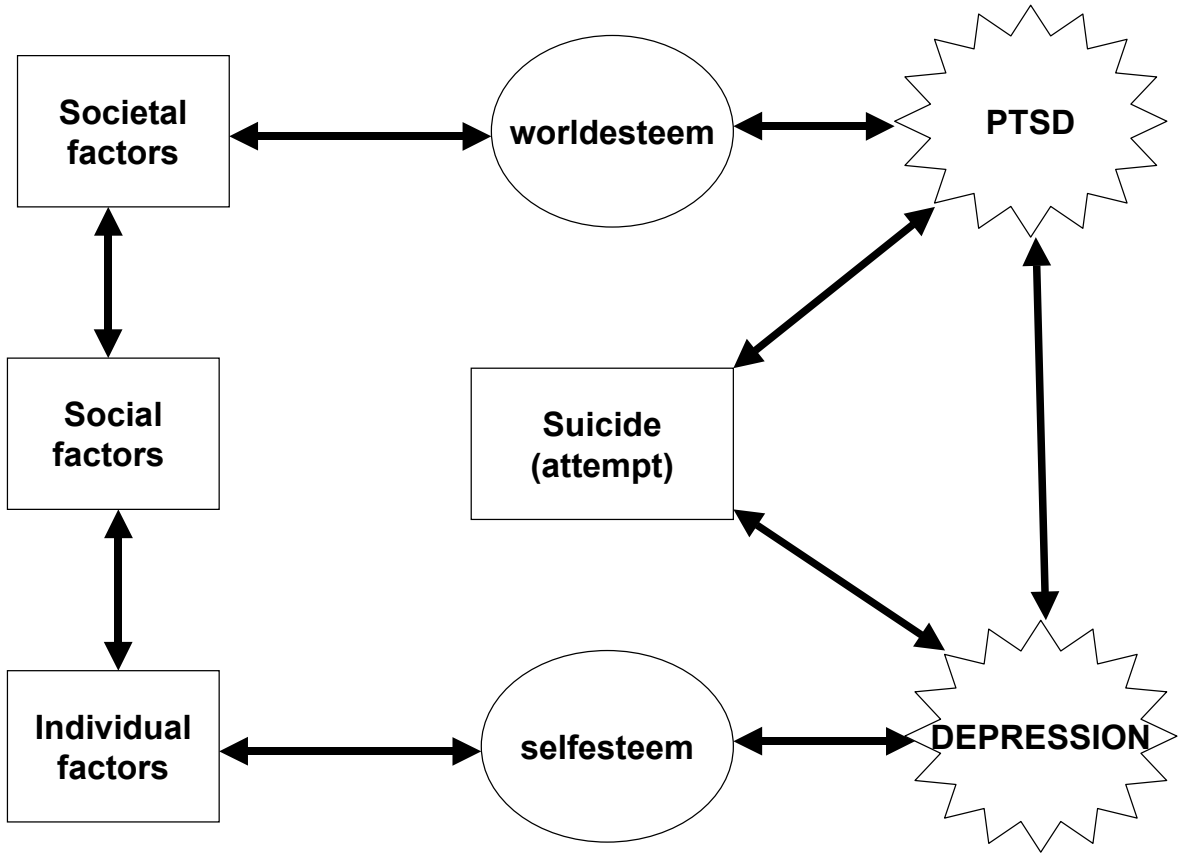

Figure 4. Model for explanation of suicide (attempt).

From figure 4 it appears that world-esteem, self-esteem, PTSD and depression are the most important predictors of suicide, which are being influenced by societal, social and individual factors. Societal factors refer to the recognition that veterans receive: the success of the operation they participated in is largely determining this recognition. Participation in a lost war most of the time means lack of recognition from society. Lots of studies on suicide among veterans regard the Vietnam War, which is an overt example of a lost war, with all its consequences for lack of recognition from society. Participation in peacekeeping operations has a similar risk of lacking societal recognition. Societal discussion about the pro's and con's of such operations endure from the very start of planning these operations and because it is an out of area operation, lack of support is to be expected. The success of peacekeeping missions is questionable most of the time. So peacekeeping operations might present their own risk factors in suicide (Mehlum1995, Mehlum 2001).

Referring to social factors, Vietnam veterans lacked social support by the discussion about the sense of this war that ripped families and friendships. In returning home, this social support lacked very overtly, because military were being rotated individually. Only after many years and depending on the first initiatives for reunions, some sort of social support emerged. Next to this phenomenon, the culture of the armed forces prohibits to share emotions or feelings of guilt: success seems to be the only thing that really counts. This brings the small group of people who have to share their emotions as an utter attempt to survive into the position of a minority, with all risks of discrimination. From the history of less successful wars it appears that it takes time to let this group grow. In Australia it took 12 years after the last

Australian soldier left Vietnam, before a mass demonstration of more than 25.000 Vietnam veterans marched through the streets of Sydney, bringing out an emotional outcry that had not be seen in many years before. In the years after, this march appeared to be the motor for massive recognition from society for the performance, grieve and pain of thousands of veterans.

On the individual level the real questions of veterans have to be addressed tacitly. These questions, often covering strong feelings of guilt, resemble questions of survivors of disaster: 'why did I go there, why did I not leave earlier, why didn't I die', while the logic of outsiders cannot relate the presence of the victim to the occurrence of the disaster in any way.

In box 2 we describe three cases of suicide in the Netherlands armed forces. To protect the privacy of the deceased we have left out their names and changed their situations, without loosing the bare essence of the cases.

Box 2. Suicide in the Netherlands armed forces.

A soldier from an ethnic minority fails to meet the terms of his basic training and is dismissed. Afterwards he tries to get into another part of the armed forces and has to wait for the new selection procedure. In his early youth his father left him, during basic training he had lost contact with his mother. While waiting for the selection procedure he runs out of money. In this crisis he calls for the help of the social work branch of the department of Defence. An intervention by a social worker leads to payment in advance of his salary, which he uses to buy a train-ticket to visit his girlfriend. This girlfriend lives with her mother on an apartment on the tenth floor. When he arrives he has to wait there for his girlfriend, who shows up a little later. After a strong dispute with his girlfriend he looses control of the situation and jumps down from the tenth floor…

After his initial military training, a soldier attends a technical course for heavy vehicle repair. This course is very individualistic: students do not meet for classical education at all. Therefore group-support in this course is minimal. One morning the school that offers this course is shattered by the news that on the evening before, the soldier jumped from the roof of his apartment on the eighth floor. On the rooftop the police, who investigated the suicide, found needles and remains of drugs. In the psychological autopsy it became clear that the mother of the soldier had died when he was only three years old. His stepmother lost contact when he entered the military. Within one year after the burial of the soldier, his father, who never had accepted the loss of his wife and had severe drinking problems, committed suicide by using an overdose of medicine and alcohol.

A soldier participates in peacekeeping missions of the United Nations twice in one and a half years time. During these operations he witnesses atrocities, in which women and children are being murdered. The rules of engagement prohibit interference in these terrible situations and in reporting these atrocities to his superiors, this information seems to get lost in the chain of command of the mission on stage. After the second mission the soldier turns out to have aggressive outbursts once and again. He also resigns from the armed forces. His girlfriend who supported him during both missions gets desperate and ends their relationship. Afterwards, the soldier dies in a single car accident. In the car his letter of goodbye is found …

From the cases in box 2 it appears that suicide does happen among military personnel of the Netherlands armed forces. However, quantitative data to estimate the magnitude of this problem are still lacking or only available in a very limited way. However from a human perspective we note that every suicide is a disaster and has to be prevented whenever possible. Therefore, from these cases and from the model for predicting suicide in figure 4 we have constructed a model for prevention of suicide, as shown in figure 5.

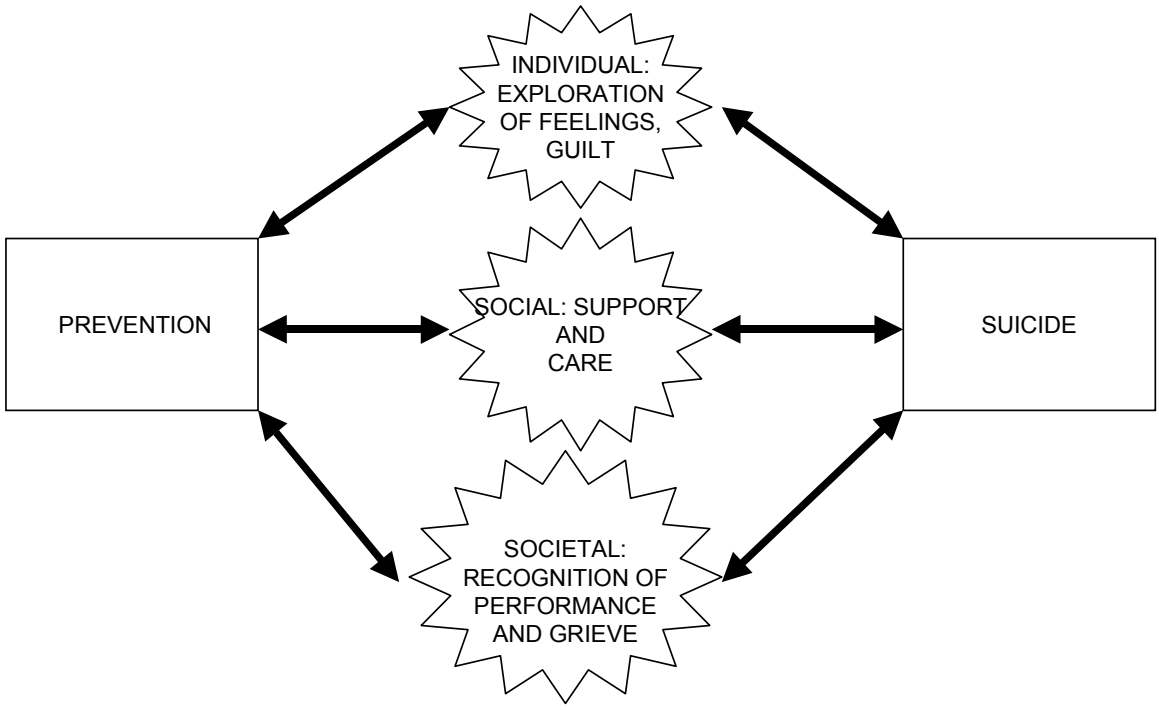

Figure 5. Model for prevention of suicide.

From figure 5 it appears that prevention of suicide on the individual level has to be realised by professional help. In helping veterans at this level, they learn to explore their own feelings in peeling off these feelings from every aspect of the events they experienced, as shown in figure 3. On the social level, most of the time there are enough sources of support and care: by parents, partners, friends and so on. However, when these sources are lacking, the likeliness of PTSD to emerge increases, and also the likeliness of suicide. In research among Gulf-war veterans the loss of a partner or friend turned out to be strongly related to the emergence of PTSD (Miller, 1991). So partners, friends and colleagues can play an important role in the prevention of PTSD and suicide among veterans.

The societal recognition of the performance and grieve of veterans is important in preventing suicide. In the case of a war that has been won or an operation that has met its goals, veterans get this recognition in a quite natural way. However, recognition for performances or grieve of veterans in a lost war or an unsuccessful operation is hard to get. As on the individual level, this kind of prevention of suicide on a societal level appears to be an important task for professionals in veterans-organisation. In the last section of this paper we will describe how these tasks can be incorporated in policy on care for personnel of the department of Defence as well as veterans-organisations.

4.0. Data on suicide of veterans and military personnel

4.1 Cases from the United States Armed Forces

Box 2. Self-harm and violence after deployment in the war on terrorism in Afghanistan.

On June 10 2002, Sergeant First Class Rigoberto N., a member of the Third Special Forces Group who had returned from Afghanistan two days earlier, shot his wife and then himself. He had requested leave from duty in Afghanistan to resolve personal problems.

On June 29 2002, the wife of Master Sergeant William W. of the 96th Civil Affairs Battalion, a Special Forces Unit, was strangled. Wright, who had been back from Afghanistan about a month, was charged with murder.

On July 19, Sergeant First Class Brandon F. shot and killed his wife and then himself, according to investigators. Floyd was identified as a member of the Delta Force, a crack anti-terrorism unit, whose existence is not officially acknowledged.

Washington Post, July 27, 2002, page A03.

4.2 Data from the Norwegian Unifil Study

More than 15.000 Norwegian veterans participated in the Un the United Nations Interim Forces in Lebanon (UNIFIL) from 1978. Weisaeth (1994) reconstructed numbers of natural deaths and suicidal deaths among these veterans that are presented in table 1.

Table 1. Observed and expected number of deaths among Norwegian Unifil veterans up until 31st of December 1991 (Weisaeth 1992).

GROUP/ MORTALITY	Expected in Norwegian population	Observed in Norwegian Unifil veterans
Natural Deaths	113	69
Suicides	32	46
Total	145	115

From the numbers in table 1 Weisaeth concluded that the suicide rate among Norwegian UNIFIL veterans exceeds the suicide rate among Norwegian males of the same age by large. Weisaeth explains this high suicide rate among Norwegian UNIFIL veterans by the problems they have to face in working through their experiences of the deployment and in finding an appropriate place in Norwegian society after deployment. He also considers the possibility that active duty personnel might have an higher suicide rate than civilians as well, for instance because of a more risk-taking life style among military personnel in general. However, he has not studied this alternative explanation of the results in his research.

4.3. Data from the United States Armed Forces

In the spring of 1996, the United Air Force most senior leaders sensed that the details of far too many suicides were crossing their desks in daily reports of major events (Litts, 2001). In May of that year, the suicide of Admiral Jeremy Boorda, the top ranking officer of the United States Navy, caused them to take an even closer look. A team of military representatives and mental health professionals established several epidemiological baselines:

- In the first half of the 1990´s, suicide has been responsible for 24 % of all deaths
- The rate of suicide had risen significantly for enlisted males in the years preceding 1996, though still about 40% less than the age, sex and race matched civilian population.
- Fewer than one third of the suicide victims had accessed Air Force mental health services before their deaths.

The team agreed on the following three themes in problems and solutions, involved in suicide:

- Airmen feared losing their jobs and avoided seeking professional help because of the stigma associated with mental health problems and their treatment.
- Many airmen perceived that commanders and supervisors routinely viewed mental health records, which reinforced the barriers due to the stigma.
- The Air Force was losing one of its defining qualities, a supportive interconnectedness.
- In the entire constellation of risk factors, problems with relationships, the law and finances played a part in an overwhelming majority of suicides.

Therefore the team developed a prevention program, which reinforces the next three protective factors:

- Individual coping skills
- Social support and interconnectedness
- Cultural norms that promote and protect responsible help seeking behaviour.

After the intervention programme, in which a dynamic cohort of more than 5 million military personnel participated, the suicide risk was reduced with 33 % (Knox et al, 2003). Also reductions in accidental deaths, homicide, and family violence were observed.

In October 2003 it appeared that among the 130.000 US soldiers in Iraq there had been 13 suicides in 7 months, which makes a suicide ratio of more than 17 per 100.000 per year. This ratio is 49 per cent higher than the suicide ratio for US military personnel in 1992-2001, which is 11.5 . For the Secretary of Defence of the United States this high ratio was the reason to send a team of mental health care professionals to Iraq to investigate the reasons for this high suicide ratio and prevent other suicides (Ritchie, 2003).

4.4 Data from the Dutch Armed Forces

From 1978 until 1985 more than 8000 Dutch military personnel served in UNIFIL. From 2001 we are preparing a comprehensive study on UNIFIL veterans. In order to analyse the data on suicide among Dutch UNIFIL veterans, we have been collecting the data of active duty personnel of the Netherlands Armed Forces. We received data on mortality of active duty personnel of the Royal Netherlands Navy and Army in June 2002. Figure 6 presents the data on mortality of military personnel of the Royal Netherlands Navy and the Royal Netherlands Marine Corps.

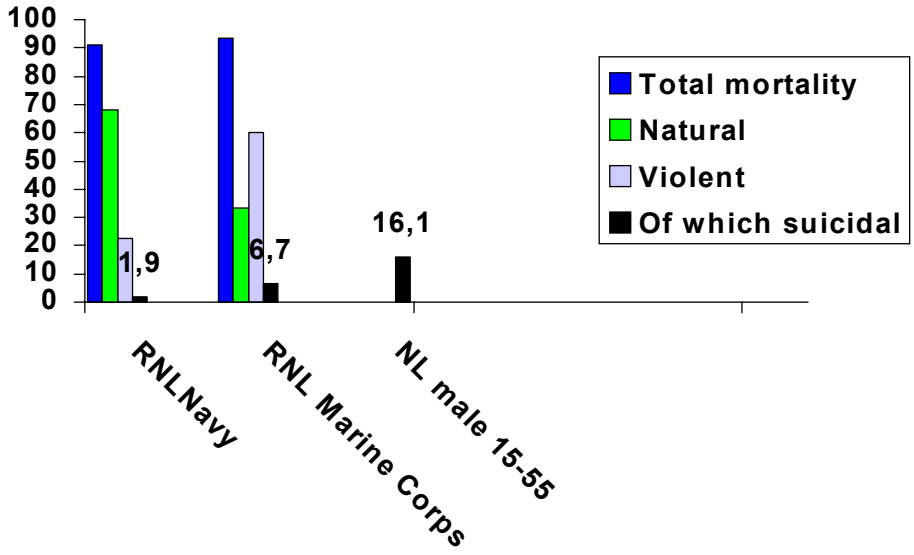

Figure 6. Mortality in active duty personnel of the Royal Netherlands Navy and the Royal Netherlands Marine Corps per 100.000 per year for 1995 to 1999, divided into natural deaths, violent deaths and suicidal deaths.

From figure 1 it appears that the overall mortality of military personnel in the RNL Navy is the same as the overall mortality of military personnel of the RNL Marine Corps. The ratio of natural deaths among military personnel of the RNL Navy is significantly higher than in the RNL Marine Corps (Chi-square=7,47, df=1, p =.006). Because overall mortality consists of natural deaths and violent deaths, the ratio of violent deaths is significantly higher in the Royal Netherlands Marine Corps. Probably, the average age of Marines is lower than the average age of Navy personnel, which can account for these significant differences.

From 1996 to 2002 the Royal Netherlands Army lost 335 people, civilians included, by natural and violent deaths. In military personnel only, there were 24 suicides in this period, which makes a ratio of 14.1 per 100.000 per year. According to the Central Bureau of Statistics in 1999 this ratio for men, aged 15-55, was 16.1. The ratio of suicide among Dutch females, aged 15-55 is considerably lower, 8.2 per 100.000. The gender-ratio of women in the Dutch Army is approximately 8.5 per cent, which makes the reference ratio of suicides 15.4, so the ratio among military personnel of the Royal Netherlands Army is lower than in the civilian population, matched by sex and age.

Almost half of the military personnel of the Royal Netherlands Army has a fixed-term contract (FTC) for two or four years. Between 1996 and 2002 15 persons of the FTC group passed away by suicide. Among this FTC group, the suicide ration was 17.9 per 100.000 per year. Military personnel from this group are honourably discharged at the average age of 30. The suicide ratio in the Dutch population, matched by this age and sex is 10.8. Compared to this ratio of 10.8, the suicide ratio among FTC military personnel of the Royal Netherlands Navy of 17.9 exceed this ratio by far.

Figure 7 shows the overall mortality of regular and FTC military personnel of the Royal Netherlands Army, divided in natural deaths and violent deaths, including suicides.

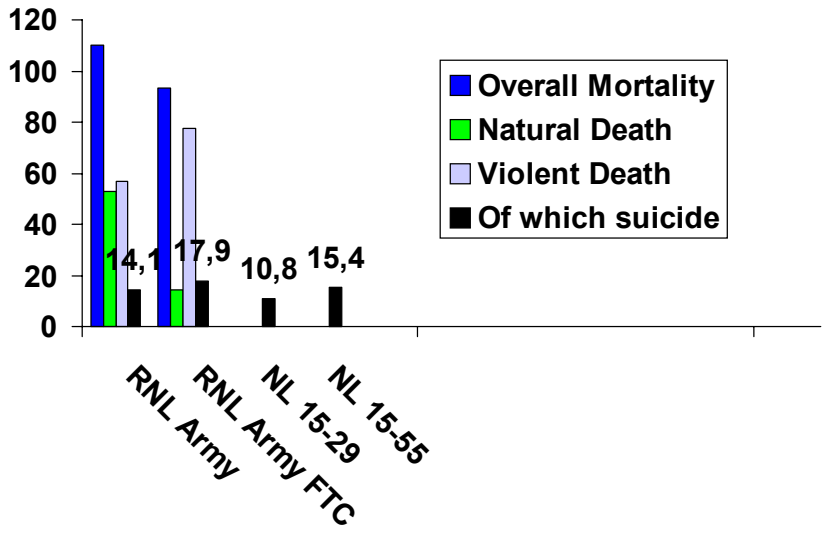

Figure 7. Mortality of military personnel of the Royal Netherlands Army, of Fixed Term Contracts, and of Dutch civilian populations, matched by sex and age from 1996 to 2002 per 100.000 per year.

From figure 7 it appears that natural deaths among all military personnel of the Royal Netherlands Army exceed the natural deaths among FTC personnel. The average age of TFC military personnel is expected to be considerably lower and might explain this difference. The suicide ratio of FTC military personnel seems to exceed the suicide ratio of Dutch civilian populations, matched by sex or age by far. Explanations for this higher ratio are still lacking, while low numbers and incidences have been criticised. There are other selection criteria for FTC personnel, and FTC personnel comes from broken families more often (Schoeman, 1995). Also difficulties in finding civilian jobs after a military career and the lack of perspective might explain this higher suicide ratio of FTC personnel, because unemployment is a risk factor for suicide (Clarke et al 2003).

Table 2 shows the expected and observed numbers of deaths in Dutch population and Royal Netherlands Army FTC military personnel per 100.000 per year from 1996 to 2002.

Table 2. Mortality ratios in Dutch population of males, aged 18-29 years and Royal Netherlands Army Fixed Term Contracts military personnel per 100.000 per year. Source: Annual Statistics Central Bureau of Statistics 2003, p.26 and p.100.

GROUP/ MORTALITY	Ratio in Dutch population	Ratio in Royal Netherlands Army Fixed Term Contracts
Natural Deaths	35	14
Suicides	11	18
Total	46	32

From table 2 it appears that the ratio of natural deaths of FTC personnel is lower than in Dutch population of the same sex and age. This might be due to a ´healthy worker effect´. The suicide ratio in FTC

personnel is higher than in Dutch population of the same sex and age. Due to small numbers, the variance in suicide numbers per year is high. Smoothing the averaged numbers per year by averaging 0 suicides in 1997 and 4 suicides in 2000 reduces this variance. In that case, a Student-t-test shows a significant difference between observed and expected numbers of suicides ($t = 2.38$, $df=6$, $p=0.027$). A Chi-square test on the raw averaged numbers shows also a significant difference (Chi-square= 8.4, $df=1$, $p=.005$). Further research is needed to find valid explanations in order to make prevention programs work.

5.0 Implications of research, models and data for military mental health care

Research among Vietnam veterans and other veterans shows that PTSD is a risk factor for suicide (Drescher et al, 2003), as well as shame and guilt. The research among Canadian veterans of peacekeeping missions demonstrates suicide ratios in military populations are sometimes biased by the healthy worker effect. From the model as shown in figure 5 it appears that societal recognition of the performance and grieve of veterans can prevent suicide among military personnel on active duty and among veterans. Within the department of Defence, this societal recognition can be fostered by paying mass media attention to actions of military personnel who are serving in war or in peacekeeping operations. When this kind of societal recognition is financed by the department of Defence, this financial support itself is also a kind of recognition. This becomes even more apparent in the care for personnel, once they have left the organisation. Not only care for physical problems, but maybe even more for psychological problems, which are not always manifest immediately, and can be neglected so easily. The need for systematic registration of and research on sel-harm, violence and suicide among veterans is an example of such care for psychological problems.

Veterans-organisations can foster this psychological care for veterans by conducting research on the specific problems veterans have to cope with. The research can add important information on the specialised help that veterans need. These organisations can foster societal recognition as well by spreading information among the mass media about the position of veterans in society or by organising regional or national gatherings of veterans. In these gatherings, veterans support each other socially by their mere attendance. Also partners, friends or colleagues can join these gatherings, and support the veterans on the social level in many ways. Education of veterans, partners, friends and colleagues is also in important activity in these kinds of gatherings.

Careful attention for military personnel or veterans who suffer from PTSD is a special issue in these gatherings, as well as in general personnel policy. Because of the fact that in most operations only a small percentage of the personnel is being affected by PTSD, these people are a minority by number and therefore at risk for discrimination. The military culture seems to forbid any weaknesses, especially on mental issues and easily leads to this discrimination, even in veterans-gatherings.

This risk of being discriminated exists also for veterans of small and almost unknown operations, regardless if they do or do not suffer from PTSD.

Last but not least prevention of suicide can be aimed at in professional help for individual veterans, especially when they receive therapy for PTSD. In this kind of help feelings of guilt need careful attention.

Several groups can be identified, that are at risk for suicide. Military personnel of the United States Air Force is such a group, and an impressive suicide prevention program for this group has been developed very effectively. Military personnel of the Royal Netherlands Army with a Fixed-Term Contract is another example of such a high risk group. Further research on this group is needed to determine risk factors and to develop an effective prevention program. The other way around, this kind of research and help also can provide important information on the incidence of suicide, especially when we realise that at this individual level every suicide is one too many . Feelings of guilt or failure among therapists can easily rebuild the taboo we have discussed before and therefore have to be addressed very carefully.

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Soldier Health Initiative – MEDBASE Application

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GREAT PLAINS REGIONAL COMMAND

BROOKE ARMY MEDICAL CENTER

MedBase

"Making a difference where the difference counts!"

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

ABSTRACT

Combat Health Support (CHS) information management forward of the division rear boundary has not significantly changed since World War II. Automated medical information systems have not been developed for incorporation into the current Standard Army Management Information Systems (STAMIS). Paper requisitions, paper reports, chart boards, voice requests, and "stubby pencil" work are the primary tools used by all forward CHS personnel. These manual processes are inadequate to support the medical information and CHS needs of the Force XXI and Army, 2010 and beyond commander. This inadequate support is exacerbated as the battle space continues to expand and non-linear operations, with increased maneuver and operational tempo (OPTEMPO), become the norm. Digital enablers/digital tools are absolutely necessary to maintain a responsive CHS system, and to maintain the low died of wounds (DOW) rates seen over the last several years.

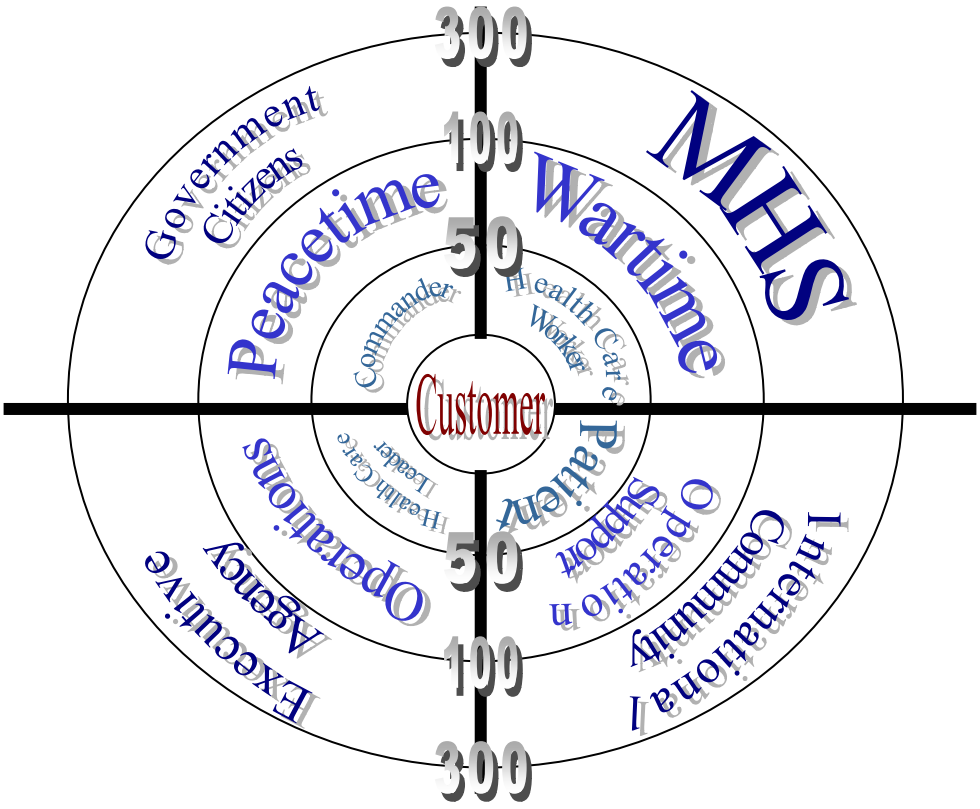
MEDBASE is the Army's interim force enterprise solution for capturing medical readiness data, to include pre- and post- deployment information and in-theatre medical tracking, and for providing medical, situational awareness to commanders. The mission is to provide users the tools necessary to efficiently perform daily business practices across multiple echelons of care and report to commanders relevant medical intelligence as a product of the normal health care practice. It automates and consolidates the entire medical readiness process (pre/post deployment, immunization, disability, medical boards, equipment, exposure etc) and provides a mechanism for tracking the health of a soldier both in garrison and in a field environment. The result is a streamlined approach to soldier medical readiness and the unprecedented collection of clinical data for decision making for commanders at all levels. MEDBASE provides a critical functionality that complements the objective system (CHCS II and CHCS IIT) under development in the TMIP architecture and serves as a bridge system to those areas currently not served by existing corporate systems, primarily those units at division level and below. This system will ensure the full range of medical readiness information is accessible years before the systems in the objective force are fully operational. This enterprise solution allows us to walk on the bridge to the future as we build it. MEDBASE is not a standalone system but works in conjunction with, and is complementary to existing corporate systems and planned MC4 hardware.

1.0 BACKGROUND

The 8 November 97 Presidential Directive specified in Public Law 105-85, National Defense Authorization Act for FY 98 states, in part, that the results of all medical examinations conducted, all health care services (including immunizations) received by service members in anticipation of deployment or during the deployment, and records of events occurring in the deployment area that may affect the health of such members shall be maintained in a centralized location to improve future access to their records. Current manual medical records systems are ineffective and frequently unavailable during deployments. Virtually all of this critical medical information is currently documented on paper, after the fact. These paper records, with their inherent bulk and vulnerability, are easily lost, destroyed, and do not lend themselves to any sort of automated screening. In order to become a part of a soldier's permanent medical record, the pieces of paper must be physically transported back to the soldier's home station and then physically placed in that record. Because of weight and storage limitations, it is impossible to maintain a high level of paper documentation during an operational deployment. Recent examples include Desert Storm, Somalia and Bosnia where immunization and treatment records lacked completeness, reliability and were frequently missing.

There is no existing automated information system to integrate and report critical medical information in support of the warfighting commander. Existing medical information systems are focused mainly on vertical information flow within a stovepipe structure in fixed medical facilities. Commanders do not have access to critical medical information that could assist them in planning operations and making decisions regarding

shaping the battle space and sustaining the force. An automated medical information system is required to link, both horizontally and vertically, the health care provider with diagnostic systems, automated treatment capabilities, evacuation platforms, other health care providers, and warfighter C2 in order to clear the battlefield and report complete situational awareness for the commander. Health care personnel at all echelons must be able to communicate with each other by audio, video, and electronic media to provide the commander with required medical information.

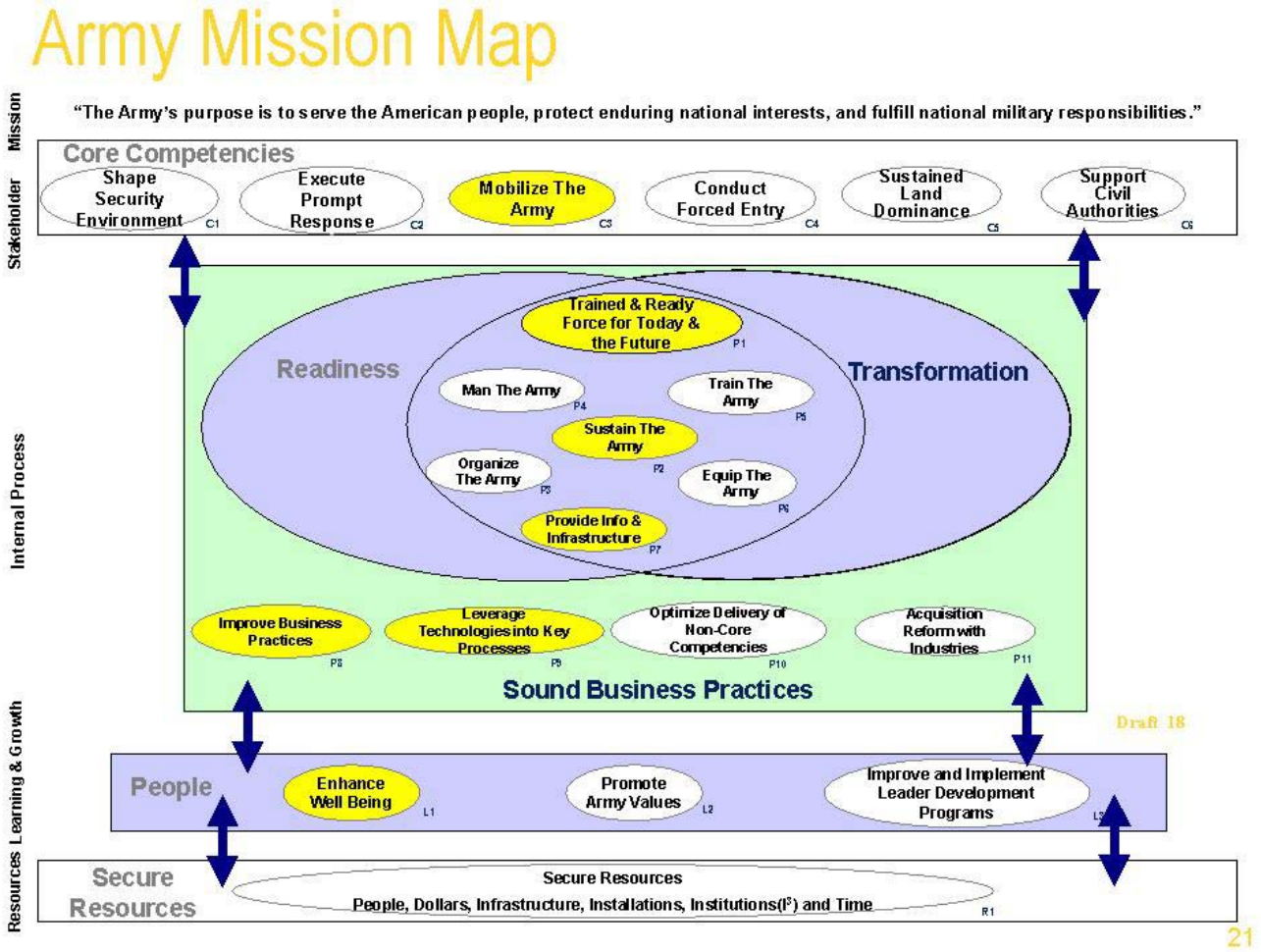


Existing practice for medical readiness is disjointed and primarily paper-based resulting in significant inefficiencies and the inability to capture critical clinical information. Those parts that are automated require multiple entries into separate systems that lack the ability to communicate with each other. MEDBASE serves as the single point for data entry and collection of medical readiness information correcting many of the problems of the existing system. It automates the entire medical readiness portion of the SRP to include pre- and post- deployment as well as the Adult Preventive and Chronic Care Flowsheet that serves as the soldier's medical record during deployments. The application is designed to operate on a network or as a standalone system in the field. Each form required by AR 600-8-101 and corresponding OTSG directives are included in the application. In addition, MEDBASE contains a profile tracking database, a robust immunization tracking database, a CHCS II compliant clinical note, and connectivity to CHCS I, the Military Health System's central clinical data repository, as well as MEDPROS. Because of the program's open architecture, it can be easily adapted to interface with existing and future systems. The program is HIPAA compliant and conforms to DoD 5200.40 and AR 25-1. Additionally, MEDBASE's electronic capture of previously paper forms along with its inclusion of more clinically relevant data fields greatly enhances a commander's ability to leverage medical readiness information for decision making. With this functionality, MEDBASE fills the information vacuum found at the division and below, linking the line to the medical community.

1.0 LITERATURE REVIEW

In regard to the implementation of information systems, there are no guarantees for success. Indeed, a few scholars estimate that between one and two thirds of information systems projects fail and of the financially draining projects, half will be cancelled for failing to meet customers expectations and overshooting the budgets (Rusin & Willimas, 2001).A review of the literature reveals several pitfalls associated with Information System implementation and suggests a number of ways that managers can act in order to successfully implement their programs. Critical success factors for information system implementation can be placed into the following categories: (1) establish a shared vision (2) plan for the entire life cycle, (3) focus on the user, (4) neutralize information system politics (Make sure you get organization buy in), (5) incorporate quality throughout the process, (6) use a team approach, and (7) implement in phases

Establish a shared vision: According to Kiely, 2002; Page, 2000. information systems fail because they aren't aligned with the organizational objectives. Page (200) asserts that critical success of any major project is directly related to how well it is linked to the organization's strategic plan. It follows then that information systems implementation must begin and be guided by a thorough understanding of the strategic direction of the organization.



Plan for the entire system life cycle. Thompson;Austin & Boxerman; Whitten & Bentley) discuss that the life cycle must contain the following 5 steps: plan, analyze, design, implement, and maintain. Planning is the step that continues throughout the entire system.

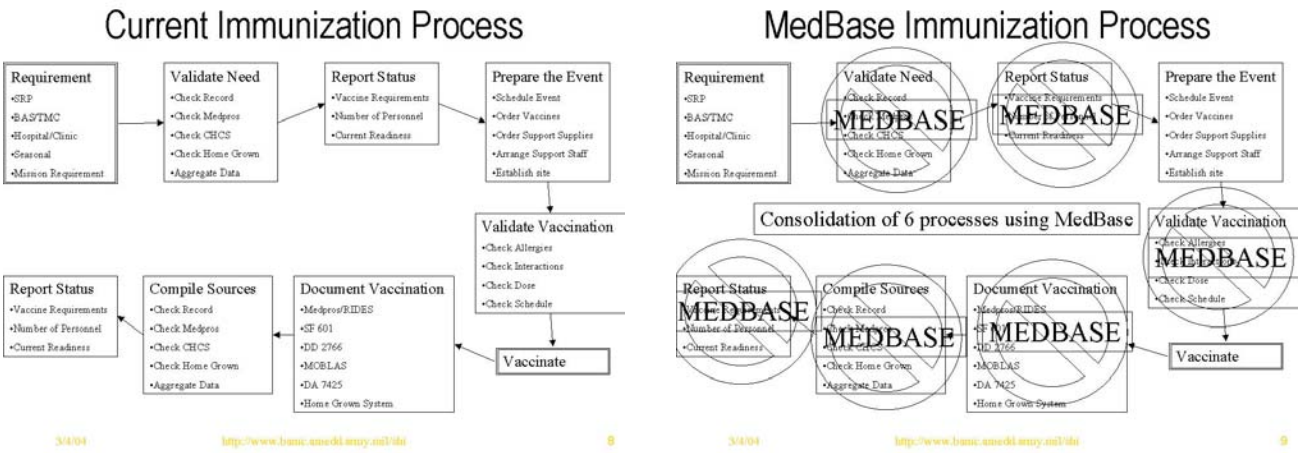
Focus on the user: This critical step is perhaps the most cited success factor in any information system implementation. The need to involve end users in every aspect of the implementation process is the linchpin to success. The literature is replete with examples of problems caused by failure to meet customer needs (Miranda, Fields, & Lund, 2001; Henderson & Deane, 1996; Treister, 1998; Rusin & Willimas; Tayntor, 1993) It is vital to involve the end users throughout the implementation process. Detailed interviews must be conducted that focus on the five W's—who, what, where, when and why (e.g. "who uses the data?" and "where does the data come from? Further, involve the user in both hardware and software selection beginning with the user interface. Regarding hardware, selection of a main frame should start with the hardware that is closest to the customer, then build upon it rather that force-fitting applications into platforms selected by just the information system team.

Neutralize Information System politics: Though failure to meet customer needs ranks high among the reasons for information system project failures, organizational politics is considered to be the biggest threat to successful information system implementation (Overton & Frolick, 1996).

Incorporate quality throughout the process. Rather than treating quality as an afterthought, Rusin and Williams recommend that information system implementers weave aspects of quality throughout the implementation process and ensure that all individuals involved understand its significance towards project success (2001)

2.0 SHORTCOMMINGS OF THE EXISTING SYSTEM:

There is no existing automated information system to integrate and report critical medical information in support of the warfighting commander. Existing medical information systems are focused mainly on vertical information flow within a stovepipe structure in fixed medical facilities. Commanders do not have access to critical medical information that could assist them in planning operations and making decisions regarding shaping the battle space and sustaining the force. An automated medical information system is required to link, both horizontally and vertically, the health care provider with diagnostic systems, automated treatment capabilities, evacuation platforms, other health care providers, and warfighter C2 in order to clear the battlefield and report complete situational awareness for the commander. Health care personnel at all echelons must be able to communicate with each other by audio, video, and electronic media to provide the commander with required medical information.



Specific shortcomings include, but are not limited to the following.

(1) There are inadequate automated medical C4I systems to support warfighter planning and decision making.

(2) The CHS systems are manual at brigade, battalion, and company level. Manual information systems do not interface with emerging warfighter digital systems. Manual systems cannot support Force XXI command requirements for timely CHS information. Without timely CHS information, commanders cannot influence operations. The shortfalls and inadequacies (particularly in information exchange capabilities) of current manual information systems are exacerbated in combat and Stability and Support Operations (SASO) environments which increasingly require rapid and frequent mobility. Manual record keeping systems do not comply with Presidential and Congressional directives for soldier monitoring and health documentation.

(3) There is no interoperability between existing medical systems to enhance timely, efficient medical treatment.

(4) There are no deployable automated clinical information systems to support the warfighting commander through the entire spectrum of split-base military operations.

(5) There is no patient movement tracking system in the theater to provide the commander information about the location and status of his soldiers who have become casualties.

(6) There are no automated systems to document intra-theater immunizations, treatment, and health hazard exposures. This information is important in providing the commander with information regarding the medical readiness of his unit.

(7) There is no automated medical logistics information systems at corps and below to expedite the resupply of medical supplies to forward areas and decrease the medical logistics footprint on the battlefield by eliminating stockpiles of medical supplies.

3.0 Scope

Table 1: MEDBASE System Impact Across the Battlefield Operating Systems (BOS)

MEDBASE System Impact / Army BOS	Interoperability	Combat Readiness	Decision Making	Synchronization	Mental Agility	Clearing the battlefield	Maneuver	Visibility	Logistics	OPTEMPO	Integrated Info	Medical Anchor Desk	Rapid NBC assessment	Project, protect, sustain the force	Information Dominance	Shape battlespace	Conduct decisive Ops
Intelligence	X	X									X		X		X	X	X
Maneuver		X				X	X			X							
Fire Support		X															
Air Defense		X															
Mobility & Survivability		X	X	X	X	X	X	X		X			X	X	X	X	X
Combat Service Support	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

The MedBase system will significantly improve the Force XXI commander's ability to:

(a) Rapidly deploy a healthy and fit force by reducing deployment processing time to minutes rather than hours or days by providing automated tools for the purpose of recording, reporting, and then validating medical deployment readiness.

(b) Reduce combat mortality and morbidity by providing CHS personnel with a wealth of indexed electronic medical reference necessary to respond to the sick and wounded. Studies indicate that approximately 30% of those who are KIA have the potential of being saved through rapid, technically qualified intervention.

(c) Account for wounded/injured soldiers while in the CHS system through an automated in-transit visibility interface between the CHS system and the personnel system.

(d) Synchronize the CHS effort with the overall CSS effort by providing near real time digital CHS information on the 10 CHS functional areas through Global Combat Support System-Army (GCSS-A) or directly to CSSCS.

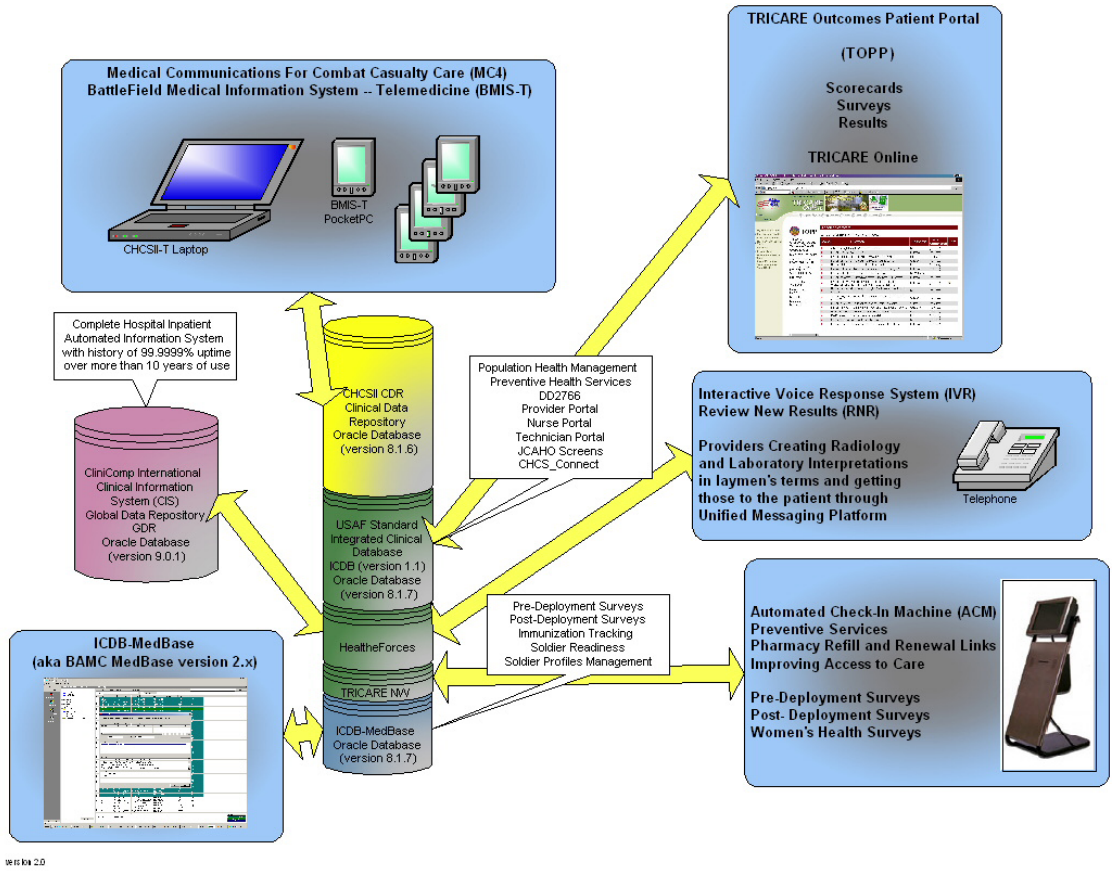
(e) Provide force health protection through trend analysis of health care encounters (diagnoses) and improved environmental and occupational health surveillance by providing command surgeons and preventive medicine experts with immediate digital access to this information.

(f) Provide commanders and medical officers the necessary medical training information necessary to support operations. This includes but not limited to mandatory life saver training for 91W to unit medical training such as Combat Lifesavers. In order to ensure the maximum qualified skill set on the battlefield, MedBase will track and report the status of necessary medical skills on the battlefield at an individual level and unit. MedBase will also assist planners to find the necessary skills for an operation. For example, find professional fillers who have tropical medicine training necessary for a deployment in the pacific basin.

(g) Transition to a joint environment due to the standardization of CHS business practices and capabilities, as well as streamlined flow of relevant medical information between the Services.

(h) The MedBase system also sets the foundation for CHS of Strike Force and Army, 2010 and beyond. The concepts outlined in this document are absolutely essential to the successful execution of the basic tenets of Army, 2010 and beyond and Strike Force warfighting concepts because of their dependence on Force XXI concepts as a foundation. As the Army moves to the future, regardless of the concept, as long as soldiers are involved, the CHS basic functions must still be accomplished. The 10 CHS functions include: Medical Command, Control, Communications, Computers and Intelligence (C4I); Medical Logistics and Blood Management; Preventive Medicine; Veterinary/Food Inspection Services; Laboratory Support/Environmental Hazards Testing; Hospitalization; Forward Casualty Resuscitation and Treatment/Area Support; Dental Health; Medical Evacuation; and Combat Stress Control support. Assured telecommunications and digital tools provide a reach back capability and development of a more streamlined/tailored approach to deployment of CHS personnel.

MAJOR PROJECT OBJECTIVES



An information system such as the MEDBASE system helps build the build the bridge to the objective CHS system to links commanders, health care providers, and medical support providers, at all echelons, with integrated medical information. The system provides digital enablers to connect, both vertically and horizontally, all ten CHS functional areas. The MEDBASE system receives, stores, processes, transmits, and reports medical command and control, medical surveillance, casualty movement/tracking, medical treatment, medical situational awareness, and MEDLOG data across all levels of care. The MEDBASE system will be developed incrementally through the rapid prototyping and the spiral development process, which will progress the system from limited functional threshold capabilities to fully integrated objective capabilities. Objectives of this system include:

1. Rapidly deploy a healthy and fit force by reducing deployment processing time to minutes rather than hours or days by providing automated tools for the purpose of recording, reporting, and then validating medical deployment readiness.
2. Reduce combat mortality and morbidity by providing CHS personnel with a wealth of indexed electronic medical reference necessary to respond to the sick and wounded. Studies indicate that approximately 30% of those who are KIA have the potential of being saved through rapid, technically qualified intervention.

3. Account for wounded/injured soldiers while in the CHS system through an automated in-transit visibility interface between the CHS system and the personnel system.
4. Provide force health protection through trend analysis of health care encounters (diagnoses) and improved environmental and occupational health surveillance by providing command surgeons and preventive medicine experts with immediate digital access to this information.
5. Provide commanders and medical officers the necessary medical training information necessary to support operations. This includes but not limited to mandatory life saver training for 91W to unit medical training such as Combat Lifesavers. In order to ensure the maximum qualified skill set on the battlefield, MedBase will track and report the status of necessary medical skills on the battlefield at an individual level and unit. MedBase will also assist planners to find the necessary skills for an operation. For example, find professional fillers who have tropical medicine training necessary for a deployment in the pacific basin.
6. Transition to a joint environment due to the standardization of CHS business practices and capabilities, as well as streamlined flow of relevant medical information between the Services.
7. Provide commanders, down to the company level, access to real-time critical medical information, such as temporary medical profiling (disability) and MEB status
8. Reduce deployment processing time by creating automated medical reports that produce efficient medical processing to include required immunizations, prescription drug needs and eyewear
9. Provide commanders the tools for increased force health protection through trend analysis of health care encounters for injury tracking
10. Integrate data from current, standalone information systems (CHCS/ MEDPROS/ MOBLAS) by packaging the data in an easy to use format (Blur the lines of TOE & TDA medical practice).Cross multiple information system domains (MTF to BAS, garrison to field)
11. Reduce data entry requirements throughout the healthcare continuum by taking information gathered at the point of care and using it to feed administrative and readiness databases
12. Provide a continuum of care by sharing data with VA through their VISTA system

MedBase is a performance-oriented system designed to assist health care professionals and technicians at the point of care. It is designed to integrate with multiple systems ranging from clinical to administrative. It's flexibility, customizability, scalability and overall performance is unparalleled. It was designed to accommodate a variety of connectivity scenarios. One can easily connect using Terminal Services, Terminal Service Web and lastly operate totally disconnected from the network.

Clinical	Administrative	Readiness
• Electronic Clinical Note • Health Care Templates • Population Health • DD 2766 (modified) • DNBI/BI • Health Care Alerts • Rad Imaging (Web) • Immunizations/Injectables • Medical Coding • Disease Manager • Patient Manager • Patient Education • Health References • CHCS Terminal • ICDB (integrated)	• Command Module (bars, flags, sick call, profiles etc) • Email Report Subscription Patient & Provider Profiling • HEDIS • Unit & Medical Training • Basic Personnel Management • Software Development Kit • Bed Manager Personal/Clinic Profiles • Ad Hoc Reports P/PHDA transfer to AMSA • Bi-directional transfer with MEDPROS • Disconnect from Network • Save record to media • Attach various files • Exports (ie PDF) • Patient Tracker	• DD 3349 (Profiles) • DD 2795 (Pre Deployment) • DD 2796 (Post Deployment) • DA 7425 (SRP checklist) • DD 2766 (modified) • DA 3180 PRP • DA 4180 (Flight) • Depleted Uranium • IMR • Medical Training • Readiness Reports • Immunizations • Medical Boards • PRP Rating/Report

The existing practice for medical readiness was disjointed and primarily paper-based resulting in significant inefficiencies and the inability to capture critical clinical information. Those parts that were automated required multiple entries into separate systems that lacked the ability to communicate with each other. MEDBASE serves as the single point for data entry and collection of medical readiness information correcting many of the problems of the existing system. It automates the entire medical readiness portion of the Soldier Readiness Program to include pre- and post-deployment as well as the Adult Preventive and Chronic Care Flowsheet that serves as the soldier's medical record during deployments.

The first version of MEDBASE consolidated several of the databases and spreadsheets into a single program with an easy to use interface. The program was developed using commercial, off-the-shelf products. Because of its ease of use and greatly expanded functionality as compared to the programs that existed, demand for the information system grew immensely.

Numerous trips and hundreds of hands on training courses for the users of MEDBASE has been necessary to encourage the use and implementation of the information system. The end result of a comprehensive implementation of MEDBASE will ensure that a full range of medical readiness information will be assessable and thereby guarantee healthy fighting force.

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(b) FM 8-10-16 ARMY MEDICAL INFORMATION OPERATIONS, 03 SEP 1998

(c) FM 8-50 PREVENTION AND MEDICAL MANAGEMENT OF LASER INJURIES, 08 AUG 1990

(d) FM 8-51 COMBAT STRESS CONTROL IN A THEATER OF OPERATIONS TACTICS, TECHNIQUES, AND PROCEDURES, 29 SEP 1994 , CHANGE 1, 30 JAN 98

(e) FM 8-55 PLANNING FOR HEALTH SERVICE SUPPORT, 09 SEP 1994

(f) FM 22-51 LEADER'S MANUAL FOR COMBAT STRESS CONTROL, 29 SEP 1994

(g) AR 40-5, PREVENTIVE MEDICINE, 10/15/90

(h) AR 40-10, HEALTH HAZARD ASSESSMENT PROGRAM IN SUPPORT OF THE ARMY MATERIEL ACQUISITION DECISION PROCESS, 10/1/91

(i) AR 40-25 NUTRITION STANDARDS AND EDUCATION, 6/15/01

(j) AR 40-501 STANDARDS OF MEDICAL FITNESS, 3/28/02

(k) HQDA LTR 40-00-1, ERGONOMICS PROGRAM, 7/14/00

(l) HQDA LTR 40-02-1, ERGONOMICS PROGRAM, 7/14/02

(m) WAC 296-62, Washington State Department of Health

(n) AR 602-2. Manpower and Personnel Integration (MANPRINT) in the System Acquisition Process

(o) 29 CFR 1960.8. Agency responsibilities. (Available from the Superintendent of Documents, Government Printing Office, Washington, DC 20402.)

(p) DODI 6055.1. DOD Safety and Occupational Health (SOH) Program

(q) EO 12196. Occupational Safety and Health Programs for Federal Employees

(r) NIOSH Publication 94-110. Applications Manual for the Revised NIOSH Lifting Equation, U.S. Department of Health and Human Services (DHHS), NIOSH. 1994 . (Available from NIOSH Publications , 4676 Columbia Parkway, Mail Stop C-13, Cincinnati, OH 45226-1998.)

(s) OSHA Publication 3123. Ergonomics Program Management Guidelines for Meatpacking Plants, U.S. Department of Labor, OSHA. 1991. (Available from U.S. Department of Labor, OSHA , 200 Constitution Ave., N.W., N3651, Washington, DC 20210.) =

(t) PL 91-596. Occupational Safety and Health Act of 1970 , as amended (29 USC 651 et seq.) TB MED 503. The Army Industrial Hygiene Program

Deployment Health Surveillance

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ABSTRACT

The Gulf War drove home the need for a comprehensive deployment health surveillance system. Gulf War health questions have resulted in controversy over potentially hazardous exposures during the deployment, the possibility of adverse affects from preventive health measures, and the role of stress in causing chronic illness. The lack of comprehensive deployment health surveillance has made it difficult to determine possible causes of adverse health effects reported by Gulf War veterans.

In response, the military health system has undergone a fundamental reorientation. Today, a bold deployment health surveillance initiative, called the Theater Medical Information Program (TMIP), is part of a layered force health protection system. TMIP is a very large information system that will integrate several service medical information systems to ensure interoperable support for rapid mobilization, deployment, and sustainment of all theater medical services in support of any mission.

TMIP is being implemented incrementally, and one of the first elements to be fielded is the Joint Medical Workstation (JMeWS), which was deployed in January 2003 to support Operation Iraqi Freedom. JMeWS is a Web based application that allows commanders and medical planners to monitor the physical well being of their service members and theater medical treatment facilities capabilities. JMeWS provides the capability to view information at the theatre level or to drill down to patient records. Information collected can also be analyzed for health trends, which has already proven to be effective: Early last spring, the JMeWS system showed a sharp upward trend in service members in the Iraq theatre who were treated for combat stress. Commanders reacted by quickly sending combat stress specialists into the field to help troops cope with the stress.

TMIP development will continue through incremental deployments of subsystems like JMeWS until full TMIP implementation is achieved.

1.0 INTRODUCTION

The need for a deployment health surveillance system emerged in 1990 during Operations Desert Shield and Desert Storm. The need was reiterated a few years later when Gulf War veterans began reporting fatigue, joint pain, sleep problems and other symptoms that have not been definitively explained. Gulf War health questions have resulted in controversy over potentially hazardous exposures during the deployment, the possibility of adverse affects from preventive health measures, and the role of stress in causing chronic illness.

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

- The coordination of a joint force attack and threat of chemical and biological warfare agents emphasized the need for a common operational picture to support medical decision-making on the battlefield.
- The importance of a robust deployment health surveillance system and the myriad problems of not having one became even more apparent as the Department of Defense began investigating the possible causes of Gulf War Illnesses.
- Inconsistent patient encounter record keeping in theater, limited data on battlefield and occupational environmental exposures, and lack of pre- and post-deployment baselines made it difficult to determine the cause of adverse health effects reported by Gulf War veterans.

1.1 Deployment Health Surveillance, Part of a Layered Force Health Protection System

In response to health questions following the Gulf War and the increasing demands of a series of hazardous deployments, the military health system has undergone a fundamental reorientation.

Lessons learned from the Gulf War have driven changes in the approach the U.S. Department of Defense takes to protect the health of military forces. Today, deployment health surveillance is part of a layered force health protection system, which is a continuum of programs to maintain the health and provide multiple layers of protection to service members and their families throughout their military service.

Force health protection covers three broad areas:

- Fitness and Health;
- Protection and Prevention;
- Treatment and Care.

Medical surveillance is part of Protection and Prevention, which provides an array of medical technologies and capabilities to protect service members, including:

- Individual protective suits, masks and boots;
- Chemical and biological detection systems to alert if an agent is in the environment;
- Medical surveillance systems that provide near-real-time theater-wide information on environmental hazards, blood supply, antibiotic supply, and availability of other medical equipment.

1.2 Theater Medical Information Program (TMIP)

At the center of the U.S. Department of Defense's medical surveillance initiative is the Theater Medical Information Program, commonly called TMIP. TMIP will integrate several service medical information systems to ensure precise, interoperable support for rapid mobilization, deployment, and sustainment of all theater medical services in support of any mission.

TMIP is a very large information system, which is being fielded incrementally. Components of the system are put into service as soon as they are ready, without waiting for the entire system to be finished. When the system is complete, theater commanders will be able to track trends, take preventive action, and keep their forces fit through the TMIP's ability to collect, analyze, and use collective medical information across the services throughout the theater in near real-time. Using TMIP, commanders will be able to determine the location and health status of injured warfighters anywhere in the theater.

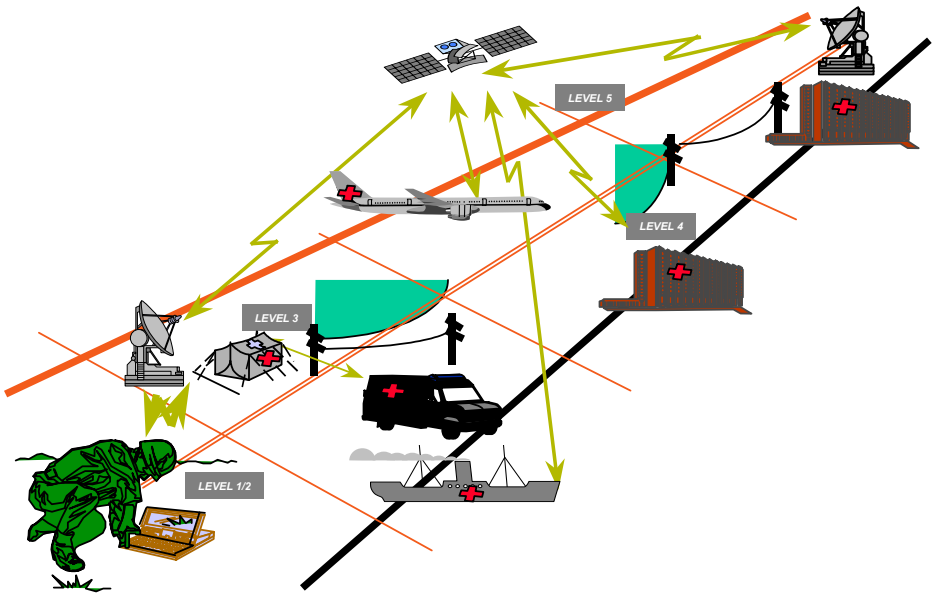


Figure 1: Medical information will be captured from lowest levels of care and will move with the patient and, in most cases, ahead of the patient through levels of care in the theater.

TMIP addresses specific deficiencies in:

- Limited health care data collection for post-operational analysis (e.g., Gulf War Illnesses);
- Insufficient interoperability between the services' medical operational forces;
- Inadequate automation for medical situational awareness;
- Lack of patient visibility (where is PFC Smith and how is he doing?).

1.3 Joint Medical Workstation (JMeWS)

The Joint Medical Workstation, called JMeWS, is an element of TMIP that was deployed in January 2003 to provide to commanders online, near-real-time medical situational awareness for forward-deployed forces during Operation Iraqi Freedom.

Information from four military medical surveillance systems is sent to JMeWS databases, where it is integrated and made widely available through classified and unclassified Internet to theater commanders, medical planners, and others who need the information.

Four applications running on the JMeWS system provide reporting, analysis and drill-down capabilities:

- Watchboard and Medical Common Operating Picture (MedCOP) provide graphical user interface to view and drill down into medical logistics and operational information;
- Medical Data Surveillance System (MDSS) and the Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE) provides information on chemical, biological, or radiological exposures, disease and non-battle injuries, and major ICD-9 Code reporting and analysis capability.

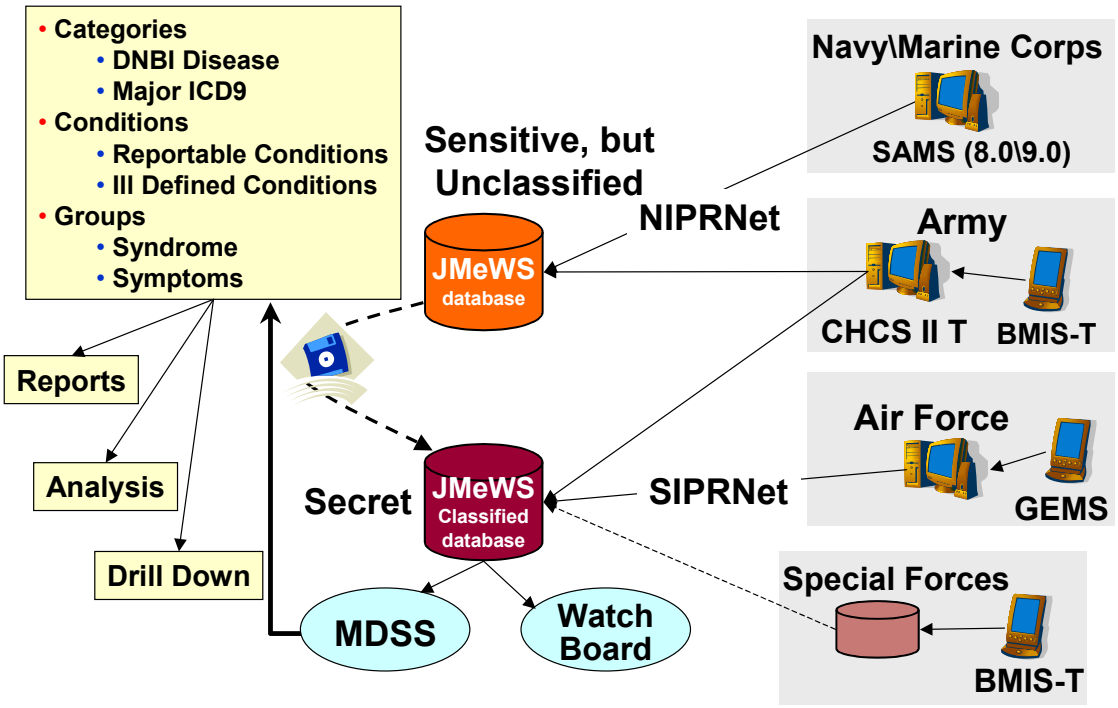


Figure2: JMeWS integrates medical surveillance information from four service systems. Applications running on JMeWS, such as MDSS and Watchboard, provide reporting, analysis, and drill-down capability.

1.3.1 JMeWS Secure Web-Based Access

The JMeWS applications suite is accessed through the Deployment Health Support Directorate's Force Health Protection portal, which is an unclassified web site accessible through the Non-Classified Routing Network (NIPERNet), that acts as a gateway to sensitive or classified information that military medical planners use in performing their mission. Access to the unclassified-but-restricted JMeWS system is made through the Force Health Protection portal's home page. Only computers operating in the dot-mil domain can see the home page, and a login and password are required to go beyond the home page. Users must register to obtain a login and password.

The classified JMeWS web site can only be accessed through the U.S. Department of Defense's Secret Internet Protocol (SIPERNet), which requires the user to have an account and special equipment.

1.3.2 JMeWS Principal Functions

JMeWS two principal functions are command and control and real-time medical surveillance.

1.3.2.1 Command and Control

When a medical treatment facility is established, designated personnel submit a joining report and various capability reports to the system through the JMeWS online reporting application. These reports include information such as location, equipment, supplies, blood, and personnel. Medical Treatment Facilities update these reports every day.

Once the report is updated, the Medical Treatment Facility has literally placed itself on the map. Theater commanders and medical planners can see where treatment facilities are located on the JMeWS map viewer, and by clicking on a facility icon, they can get an overview of the current status and capability of that facility.

Figure 3: The JMeWS Watchboard provides a graphical interface for Military Treatment Facility operational status and capability.

The status of capabilities is color-coded, so commanders and medical planners can get a status overview with a quick glance. With a click, users can drill down further and get detailed reports on the specific capabilities of each facility, such as the number of operating rooms, x-ray machines, and ground ambulances, or they can drill down further and get a report showing how much of each type of blood is on hand. The Watchboard also provides the capability of taking a macro view at the Theater, Component, Joint Task Force, or other command and control level, or the user can conduct a custom search for information.

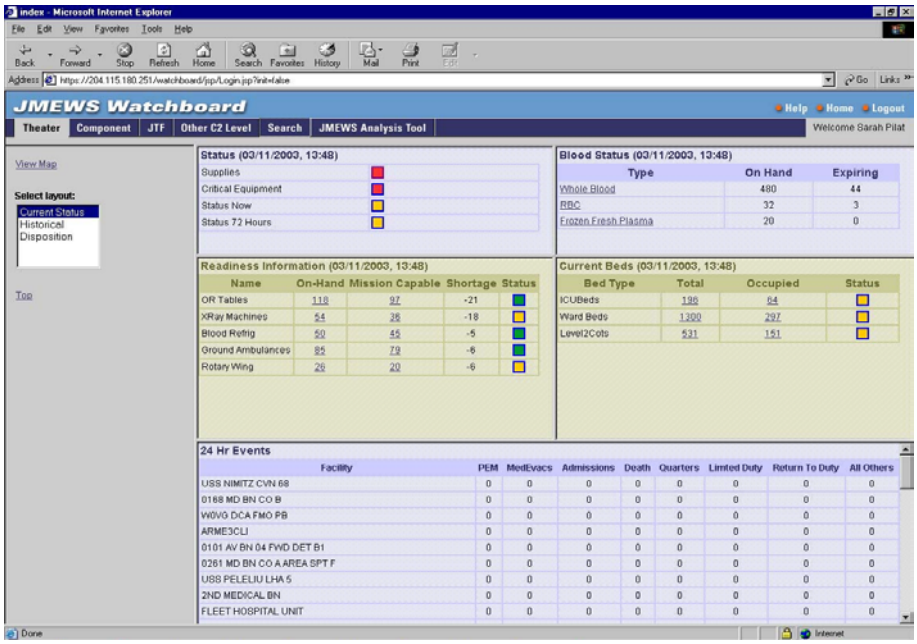


Figure 4: Medical Treatment Facility status and capability of can be viewed from the Theater down to the individual facility.

1.3.2.2 Real-Time Medical Surveillance

The second principal function of JMeWS is medical surveillance. When a patient visits a treatment facility, an electronic copy of his or her record is submitted to the JMeWS system. That, in turn, provides JMeWS with the ability to show real-time patient status. So, with a click of a button, a theater surgeon can see how many patients were treated at his facilities and what their dispositions are. The system also provides users with the ability to drill down to individual patient records.

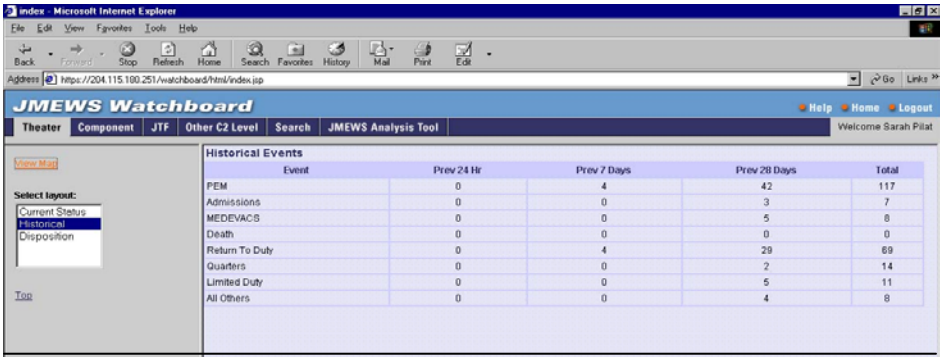


Figure 5: Patient records are uploaded to JMeWS in real time. Through the JMeWS Watchboard a theatre surgeon can get both a real-time and a historical update of patient care at his facilities.

Treatment facilities without the ability to submit the entire patient record to JMeWS use a Disease and Non-Battle Injury report, commonly called a DNBI report, to indicate the number of patient visits to that particular facility.

1.3.3 Analysis Capability

Several JMeWS applications analyze data derived from patient records and DNBI reports looking for abnormalities. These applications use historical data in the JMeWS system to establish a baseline. When new data comes in it is compared to the baseline to determine if the new data shows an abnormal trend. If the new data is beyond parameters established for the normal range, the system will issue an alert.

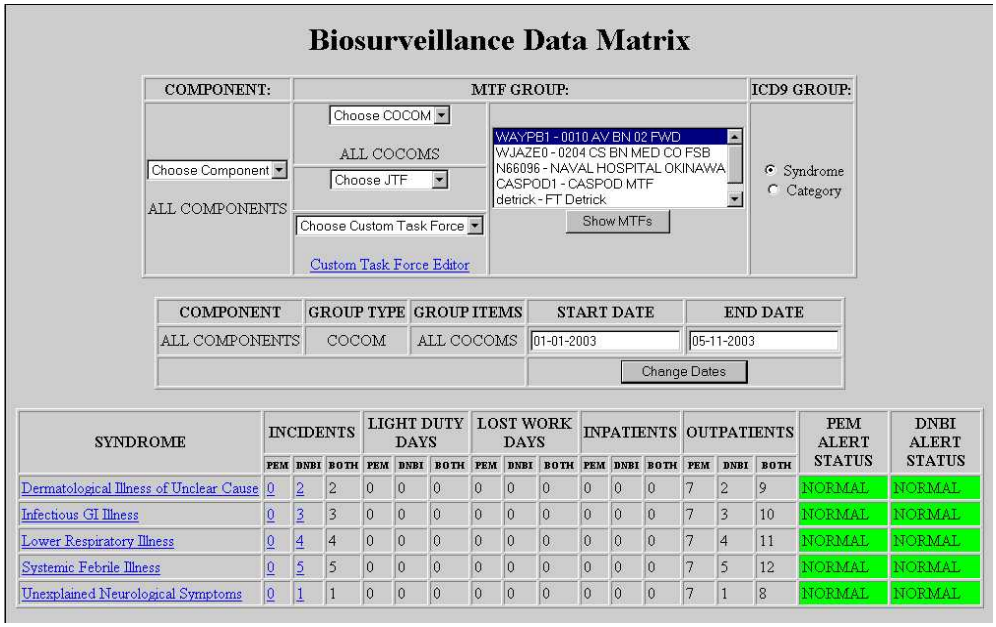
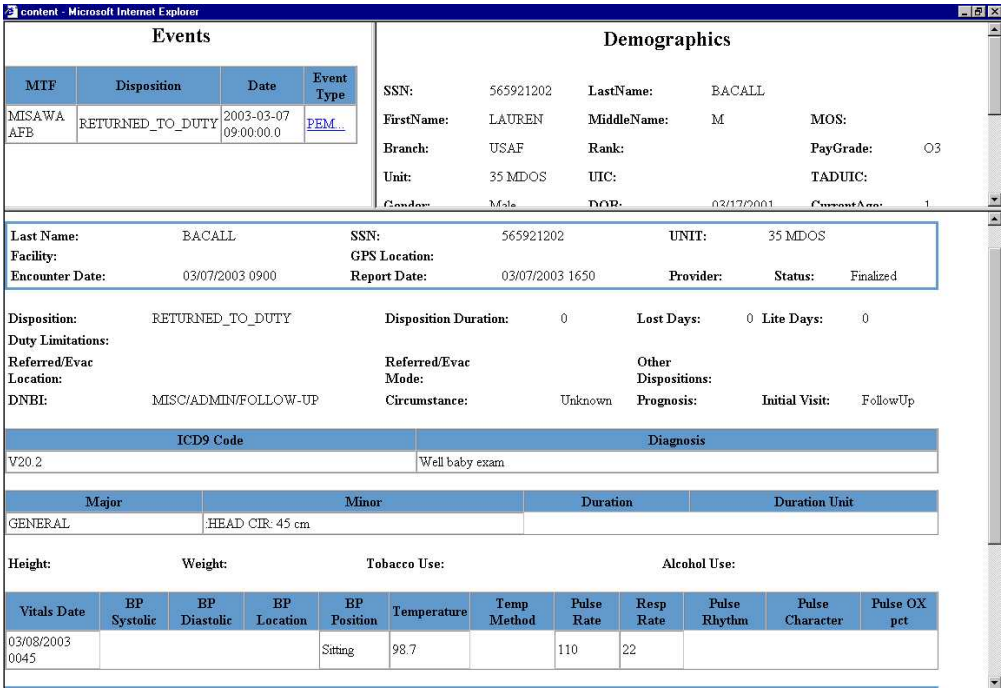


Figure 6: If new data is above parameters set for the normal range, the system will issue an alert. The JMeWS system posted an alert because of an increase in the number of reported combat stress of at the end of fighting in Iraq last spring, which prompted commanders to dispatch combat stress specialists to the theatre to treat service members affected by combat stress.

If the JMeWS surveillance tools alert for spikes in particular areas, the command surgeon can investigate the possible causes of the spike by searching the database for patients with particular symptoms. If the spike was caused by an environmental exposure, the information in patient records may determine where or how an exposure occurred.

Additional reports provide users with the capability to drill down even further, to get details behind reports such as the Chemical, Biological and Radiological, DNBI, and the Major ICD-9 Code Reports.



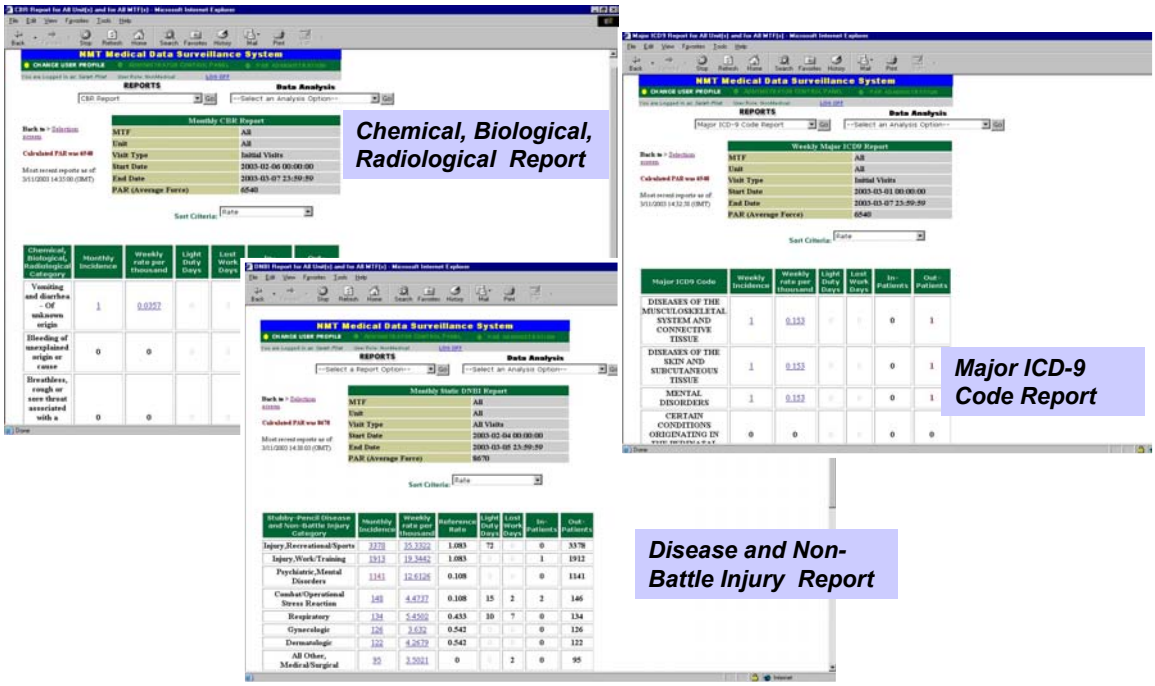


Figure 7: Individual patient records and other reports provide information for further analysis into the possible causes of alerts.

2.0 NEXT STEPS

We are planning for transition and implementation of JMeWS into the full TMIP suite while simultaneously accelerating the incremental implementation of TMIP applications like JMeWS. And to ensure the continuous flow of information while the system is in development, care must be taken to maintain interfaces with current Service systems until the TMIP system is fully implemented.

There will be a considerable information influx as the systems that form TMIP evolve. An impact study must be conducted to ensure that the system's ability to handle data evolves in concert with the growth in data.

SYMPOSIA DISCUSSION - PAPER 9

Authors Name: Mr Denicola (US)

Discussor's Name: Capt (RNL)Hovens (NL)

Question:
Are there programs where Non-US nationalities can join because peace keeping/ peace enforcing operations consist of multi-nationalities? They will cause "blind spots" if not joined.

Author's Reply:
There are systems in development that will be able to collect this data.

Authors Name: Mr Denicola (US)

Discussor's Name: Prof. Dr Von Restorff (GE)

Question:
1) How long does it take to enter all the information into the system?
2) Would that time be taken away from the patent-care-time or do you need additional personnel?

Author's Reply:
1) The patient encounters are not a problem with system speed.
2) The web pages can be a problem, based on bandwidth.

Authors Name: Mr Denicola (US)

Discussor's Name: Prof. Dr von Restorff (GE)

Question:
How much effort did you put into the software – ergonomics of your system?

Author's Reply:
The user testing defined the screens so there has been no formal operational testing for human factors to date.

Enhancing Influenza Surveillance Using Electronic Surveillance System for the Early Notification of Community-Based Epidemics (Essence)

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ABSTRACT

INTRODUCTION Influenza is a cause of preventable morbidity and mortality; timely analysis of surveillance data may allow earlier recognition of outbreaks, potentially including those caused by new influenza strains. Influenza-like Illness (ILI) is of particular interest in surveillance because many biowarfare and bioterrorism agents cause flu-like syndromes. RATIONALE The Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS) sponsors two programs, ESSENCE (The Electronic Surveillance System for the Early Notification of Community-based Epidemics) and the DoD Influenza Surveillance Program, that could assist in influenza outbreak detection and response. ESSENCE utilizes military beneficiary population outpatient visits data to detect aberrations in daily counts of ICD-9 based syndrome groups. Begun in 1999 in the Washington, DC area and expanded in 2001 following the events of September 11, this system analyses outpatient visit data across DoD military treatment facilities (MTFs), in the US and abroad. A prior study assessed the value of ESSENCE data in detecting yearly influenza activity by comparing it to a traditional influenza surveillance system used by the Centers for Disease Control and Prevention (CDC). Results of that study showed similarity between the rates of ILI visits to sentinel physicians in the South-Atlantic region and military facilities in the National Capital Area. METHODS & RESULTS Soon after September 11, 2002, ESSENCE began receiving outpatient data from all US MTFs in the world and making syndromic surveillance data easily available throughout DoD installations. Utilizing an ICD-9 code group chosen to represent ILI, the ESSENCE structure and graphic user interface and a database developed for this purpose, we developed an automated daily graphical display showing the ILI rate (proportion) graphed over time in weeks with an appropriate numerator (ILI) and denominator (outpatient visits for primary or urgent care) for each MTF. These data were also aggregated and displayed regionally, using CDC regions and three additional non-CDC regions. These data were available through the ESSENCE infrastructure in near real time, being refreshed three times per day. This new source of surveillance information was analysed, compared to other DoD and US data, including laboratory data, and incorporated into the DoD Influenza Surveillance Program weekly surveillance report. CONCLUSIONS the data show correlation of ILI activity in the military communities with CDC sentinel physician-detected activity regionally and with influenza positive cultures. Near real-time analysis and reporting, by MTF, region, and DoD, of ILI activity was accomplished. An unexpected benefit occurred during the SARS outbreak; it was useful to examine ILI rates for adverse trends and stimulate increased local public health scrutiny when indicated. Negative information, i.e. no adverse trend, was of value to local and higher authorities in assessing and communicating SARS risk to their populations.
(Disclaimer: The material in this abstract reflects the views of the authors and should not be construed to represent those of the Departments of the Army, Air Force or Navy or the Department of Defense).

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

1.0 INTRODUCTION

Influenza is a cause of preventable morbidity and mortality; timely analysis of surveillance data may allow earlier recognition of outbreaks, potentially including those caused by new influenza strains. Influenza-like Illness (ILI) is of particular interest in surveillance because many biowarfare and bioterrorism agents cause flu-like syndromes.

2.0 RATIONALE

The DoD operates the Global Influenza Surveillance Program through Air Force executive agency with support from the DoD Global Emerging Infections Surveillance and Response System (GEIS). Another GEIS sponsored program is ESSENCE, the Electronic Surveillance System for the Early Notification of Community-based Epidemics. ESSENCE had been a developmental program for syndromic surveillance using data from the National Capital Area, but following the events of September 11, 2001 ESSENCE began receiving data from all military medical facilities in DoD. The global influenza surveillance program is primarily laboratory-based surveillance while ESSENCE is syndromic surveillance. Both are high-priority programs for GEIS that could assist in influenza outbreak detection and response. Previously ILI information, when it was collected in DoD facilities, was primarily used for local surveillance and not summarized for timely use above the local level. Laboratory information from DoD could be shared and compared with national and global data, but ILI could not. In early 2002 it appeared to be technically feasible to modify ESSENCE in order to obtain timely ILI data from all medical visits in DoD. As part of this conference COL Kenneth Cox discusses how this initiative has enhanced DoD influenza surveillance; this presentation focuses on the methods used and how this was achieved.

Experience with syndromic surveillance is growing in the US and elsewhere – ESSENCE is one such system using DoD health administrative data from ambulatory medical encounters. ICD-9 coding is used DoD-wide for this purpose. The methodology for ILI surveillance focusing on ESSENCE data, aided by DoD laboratory and national influenza surveillance data, is the aim of this study. A prior study[1] assessed the value of ESSENCE data in detecting yearly influenza activity by comparing it to a traditional influenza surveillance system used by the Centers for Disease Control and Prevention (CDC). Results of that study showed similarity between the rates of ILI visits to sentinel physicians in the South-Atlantic region and military facilities in the National Capital Area. Subsequent to developing the ILI capability in ESSENCE described here, two presentations, Foster, et al[2] and Elbert[3] at the NATO meeting on pandemic influenza planning held in 2002 in St. Petersburg, Russia presented ILI data from this method. A recent presentation by Gould, et al from the DoD global influenza program examined validation between DoD viral isolates and ICD-9 codes used by providers from ESSENCE.

To understand syndromic surveillance using ESSENCE, it is helpful to show how it is displayed for the user. Shown in figures 1-2 are two views from ESSENCE one sees from a typical desktop computer. The "Worldview" here is shown superimposed on the globe to add emphasis the need to view the data globally, but as seen in figure 2, one can adjust the view as needed. Each rectangle in the outer circle represents an installation (or geographic cluster) with regional groupings closer to the center. "TCR" stands for Tri-Care Region. Colors are used to automatically bring attention to alerts. Many more views could be shown, but this is beyond the scope of this presentation. A full discussion and tutorial is available at **www.geis.ha.osd.mil** (the link provided for ESSENCE).

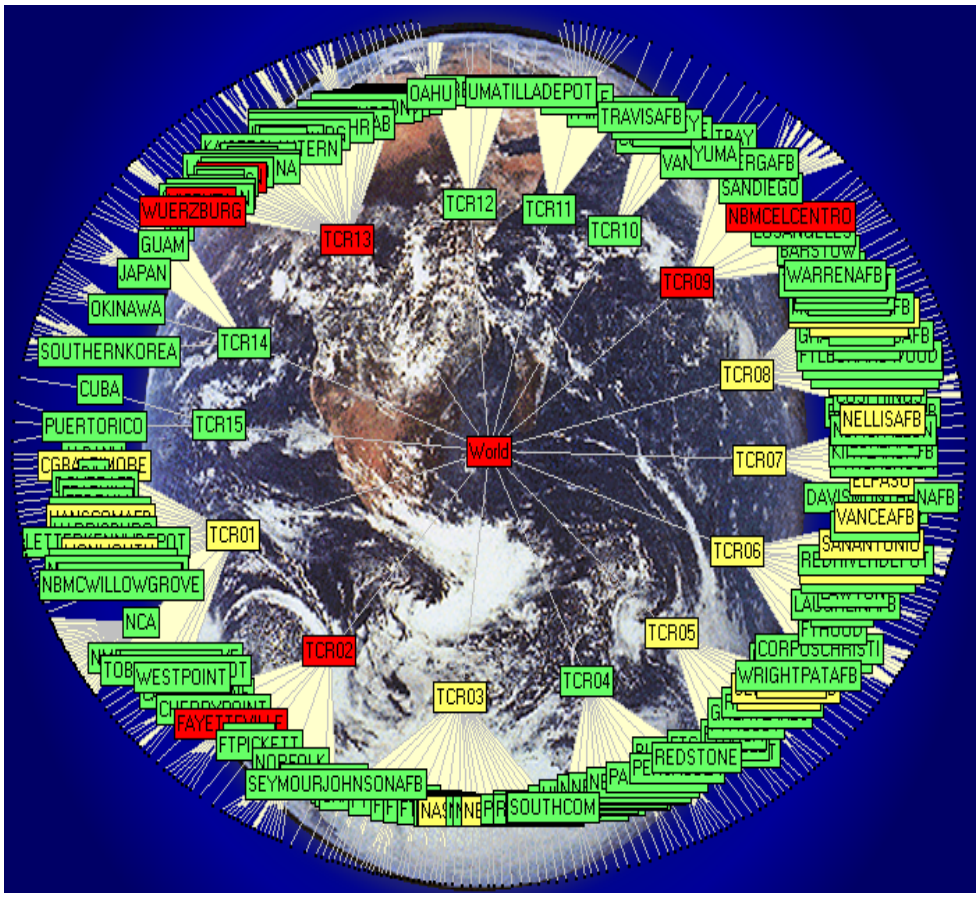

Figure 1: ESSENCE Worldview

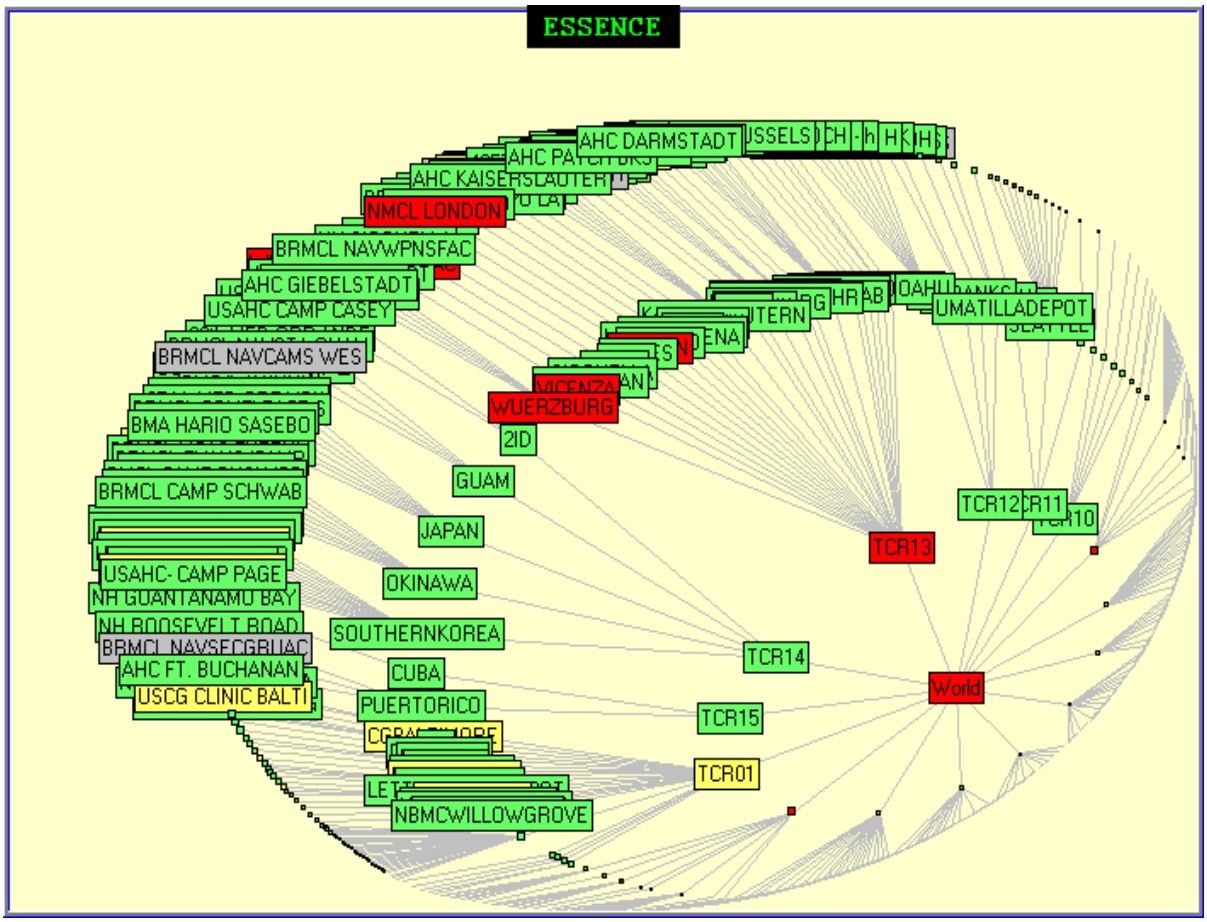

Figure 2: An "exploded" view from the Worldview shown in figure 1

3.0 METHODS

Beginning with the end result in mind, this system was envisioned as a surveillance system useful at a number of levels - military base / local area, regional and DoD-wide. Although the reason for DoD developing ESSENCE was primarily for detection of biological warfare or terrorist events, it has obvious usefulness for naturally occurring epidemics such as influenza. Also, since other national surveillance systems exist and since influenza outbreaks occur regularly, comparison and validation with US national data is possible. CDC surveillance uses four systems as shown. The sentinel physician system is based on voluntary ILI reports from a sample of physicians (about 900) across the US and reports the percentage of ambulatory patients seen for ILI, based on a case-definition. Figure 3 is an example of CDC ILI surveillance from week 1 of 2004. Figure 4 shows US laboratory-based data from the WHO/NREVSS system. Comparable data exist in DoD for both, but until recently only DoD laboratory data was readily available for surveillance. Comparison with non-US ILI surveillance data is possible as well in areas of Europe and the Pacific Rim where DoD bases are located but this has not yet been done.

Sentinel physicians (ILI)	WHO/NREVSS collaborating laboratories*
122 cities mortality	Survey (weekly) of state epidemiologists

- DoD Global Influenza Surveillance program is a collaborating laboratory

Table 1: Four methods for Centers for Disease Control and Prevention (CDC) influenza surveillance

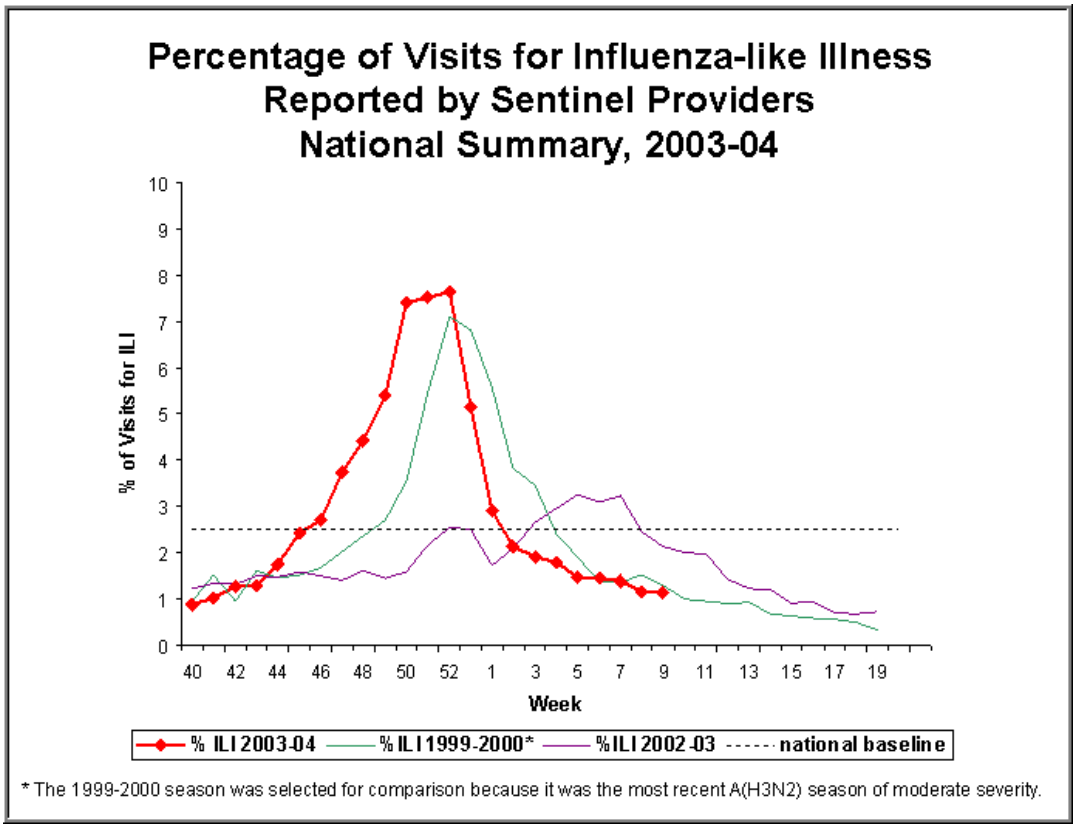

Figure 3:CDC ILI surveillance Based on Sentinel Physician in US, Week 9 2004

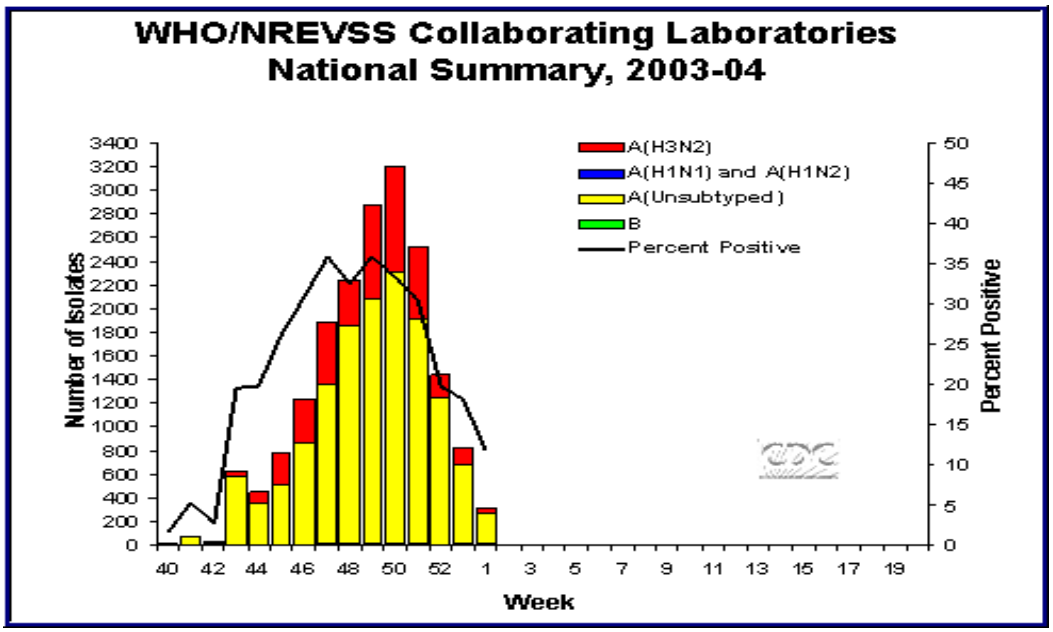

Figure 4:US Laboratory Based Surveillance, Week 1 2004

Examples of US national reporting are shown in the graphs in Figures 3 & 4 show CDC data from sentinel physician ILI system and from laboratory based surveillance. Note that each has a line graph display of percent (or proportion) over time in weeks. This actually is a cumulative proportion for the week for the ILI display. For the 2003-2004 influenza season shown, ILI peaked at about week 52 and laboratory viral isolates for influenza, as percent positive, peaked between weeks 47 and 49. 122 Cities Mortality from Influenza and Pneumonia surveillance (not shown) indicated a later peak than ILI and lab-based surveillance, indicating some lag between epidemic and mortality. Our intent was to develop ILI reporting that was automatic and would parallel that of the CDC sentinel physician system but would not use additional resources, using ESSENCE.

Soon after September 11, 2002, ESSENCE began receiving outpatient data from all US MTFs in the world and making syndromic surveillance data easily available throughout DoD installations. An ICD-9 code group was chosen to represent ILI as seen in table 2. The ESSENCE structure and graphic user interface were but modification was needed for an added capability; proportions were needed in addition to count, or numerator, data. The ESSENCE group, primarily Mr. Mansfield, modified the programming code (ESSENCE-1B version so that medical encounters resulting in these ICD-9 categories and the appropriate visits were counted; once developed this program ran three times a day to update the database developed for this purpose and it continues to do so. Data from 313 medical facilities are included; distributed according to Service as shown in figure 5. Minor modifications have been made to the ILI ICD-9 code set since inception; updated codes can be seen on the GEIS website listed above for ESSENCE and we anticipate future changes as validation proceeds.

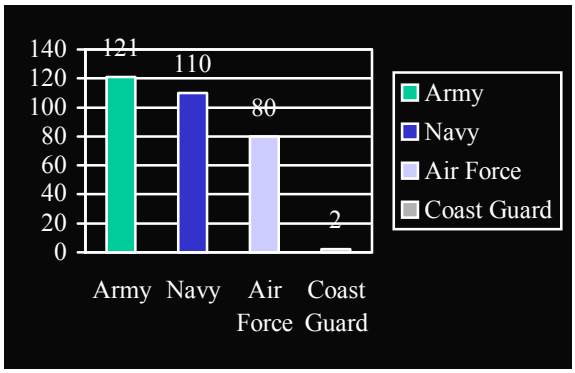

Figure 5: DoD Installations Contributing ESSENCE Data, By Service Branch

ICD-9 CODES USED FOR INFLUENZA-LIKE ILLNESS (ILI) IN ESSENCE-1B			
079.89	Viral infection NEC	466	Acute bronchitis and bronchiolitis
079.99	Viral infection NOS	466.0	Acute bronchitis
460	Acute nasopharyngitis	466.1	Acute bronchiolitis
462	Acute pharyngitis	466.19	Acute bronchiolitis due to other infectious organism
464	Acute laryngitis and tracheitis	478.9	Other and unspecified diseases of upper resp tract
464.0	Acute laryngitis	480	Viral pneumonia
464.1	Acute tracheitis	487	Influenza
464.10	Acute tracheitis w/o obstruction	487.0	Influenza with pneumonia
464.2	Acute laryngotracheitis	487.1	Influenza with other respiratory manifestation
464.20	Acute laryngotracheitis w/o obstruction	487.8	Influenza with other manifestation
465	Upper resp infection multiple or unspecified sites	490	Bronchitis not specified as acute or chronic
465.0	Acute laryngopharyngitis	780.6	Fever
465.8	Upper resp infection of multiple sites	784.1	Throat pain
465.9	Upper resp infection of unspecified sites	786.2	Cough
466	Acute bronchitis and bronchiolitis		

Table 2: ICD-9 Code set for Influenza-Like Illness in ESSENCE-1B

Data normally was aggregated in ESSENCE by installation and geographic cluster and then by logical hierarchical groupings as shown in Figures 1 & 2 (other views were available as well); we chose to add different geographic groupings so that ILI data could obtained and automatically displayed by regions used by CDC and three additional regions suitable for DoD as shown in Figure 6. Thus data from DoD facilities in these regions are organized according to CDC regions for ease of display and to facilitate comparison. Encounters included for numerator and denominator were aggregated & logically grouped by base or relevant locale. ESSENCE has 179 geographical groupings but these could be customized in the analysis to produce different local, regional and global data and graphs.

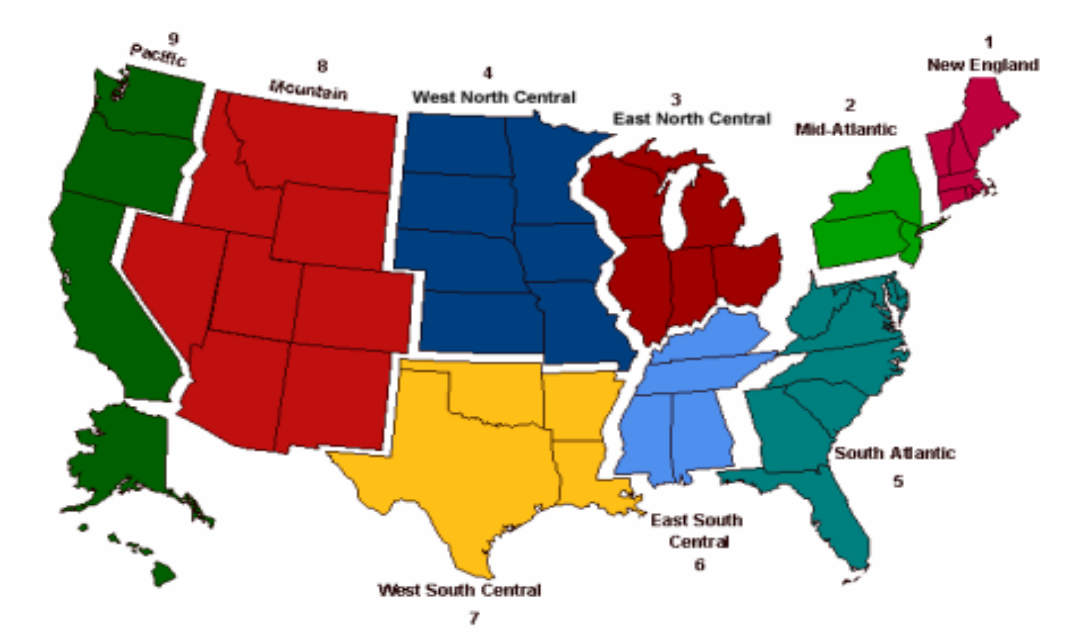

Figure 6: Regions of the US as used for influenza surveillance reporting

A cumulative weekly proportion was chosen as the best estimator of the ILI rate for the purpose of this endeavor. For the ILI proportion, the numerator data, or inclusion criteria, are medical encounters for the week coded (ICD-9) within the defined ILI code group. These syndrome groupings are a subset of "Fever" & "Respiratory" codes already used in ESSENCE. Different code sets could be used in subsequent validation or sensitivity studies, using similar methods; this flexibility was part of the original design. The denominator includes all medical encounter visits for the same time period to primary, urgent or emergency care in MTFs & medical centers. Some types of visits were excluded, for example orthopedics clinics, where they could be identified as unlikely to belong in the denominator.

4.0 RESULTS

We developed an automated daily graphical display showing the ILI rate - the cumulative proportion for 1 week - graphed over time in weeks for each MTF having standard DoD Ambulatory Data System (ADS) capability. Data are also aggregated and displayed regionally and globally. These data are made available through the ESSENCE infrastructure in near real time, being refreshed three times per day. This new type of surveillance information was analysed, compared to other DoD and US data, including laboratory data, and incorporated into the DoD Influenza Surveillance Program weekly surveillance reports.

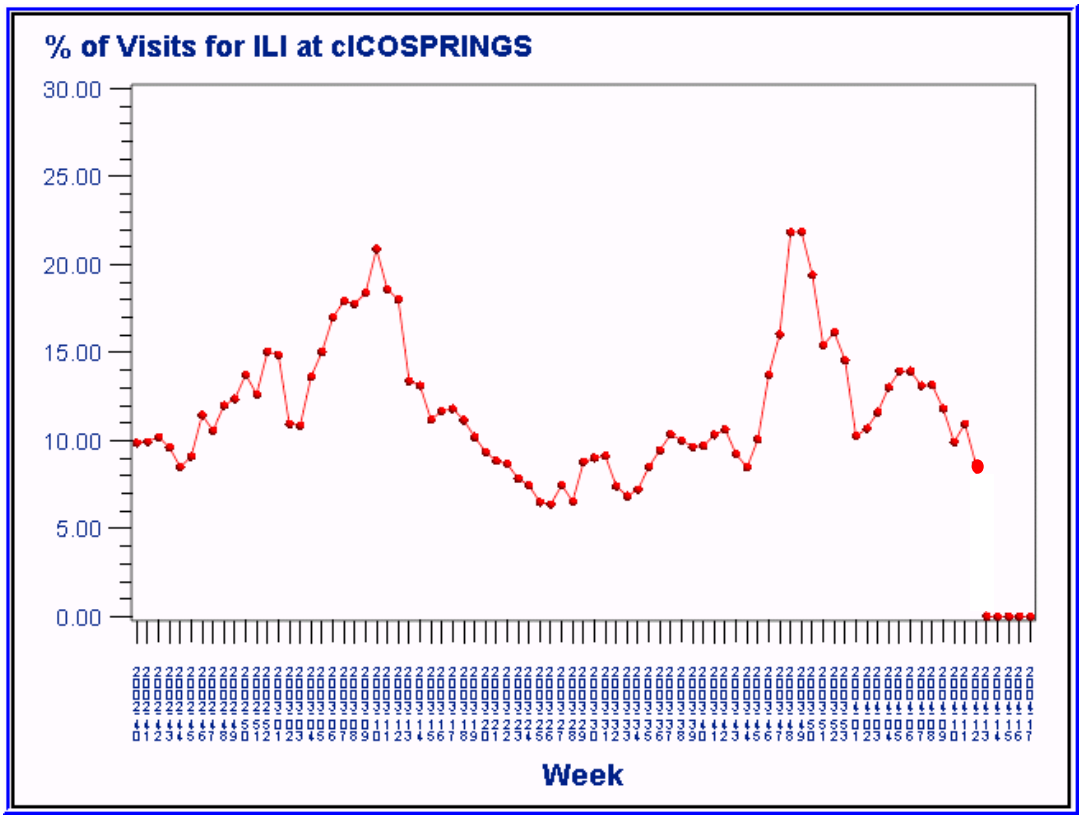

Figure 7: ILI ESSENCE graph for Colorado Springs (local) grouping, week 12 2004

The graph in figure 7 shows ILI rate over time in weeks for the Colorado Springs local area from week 40 2002 through week 12 2004, spanning nearly two influenza seasons. The peaks are consistent with reports of influenza activity in this area. A regional view is shown in figure 8 and DoD global data in figure 9 for the same period. This is the global graph that includes all available DoD data for the time period. Similar graphs are available to users through ESSENCE in near real time. This automated graph is available for bases, CDC-like regions and DoD and the data can be readily accessed. Each location will experience its own baseline level of ILI activity even in the absence of influenza. Keep in mind that that there are many illnesses that mimic flu and that potentially can be detected through surveillance. Also the magnitude of the proportions, as shown on the Y-axis has a range of 6-22% approximately and the baseline is nearly 10%. In contrast, CDC's sentinel system has a baseline below 2.5%; CDC use of a case definition and our use of an ICD-9 code set is the major reason for this difference in magnitude of ILI measurement. In addition to infectious disease occurrence, coding practices may be quite variable at different locations and influence these rates. By aggregating data from MTFs, coding variability among providers and MTFs tends to have less influence on ILI as measured. Rates for the current week will also show more variation on Monday and Tuesday than on Friday because it is the cumulative ILI proportion for that week is measured, with more data added as the week progresses. Likewise holiday periods may show unusual patterns, but in each of these circumstances, the fact that a proportion is used makes the measure more stable.

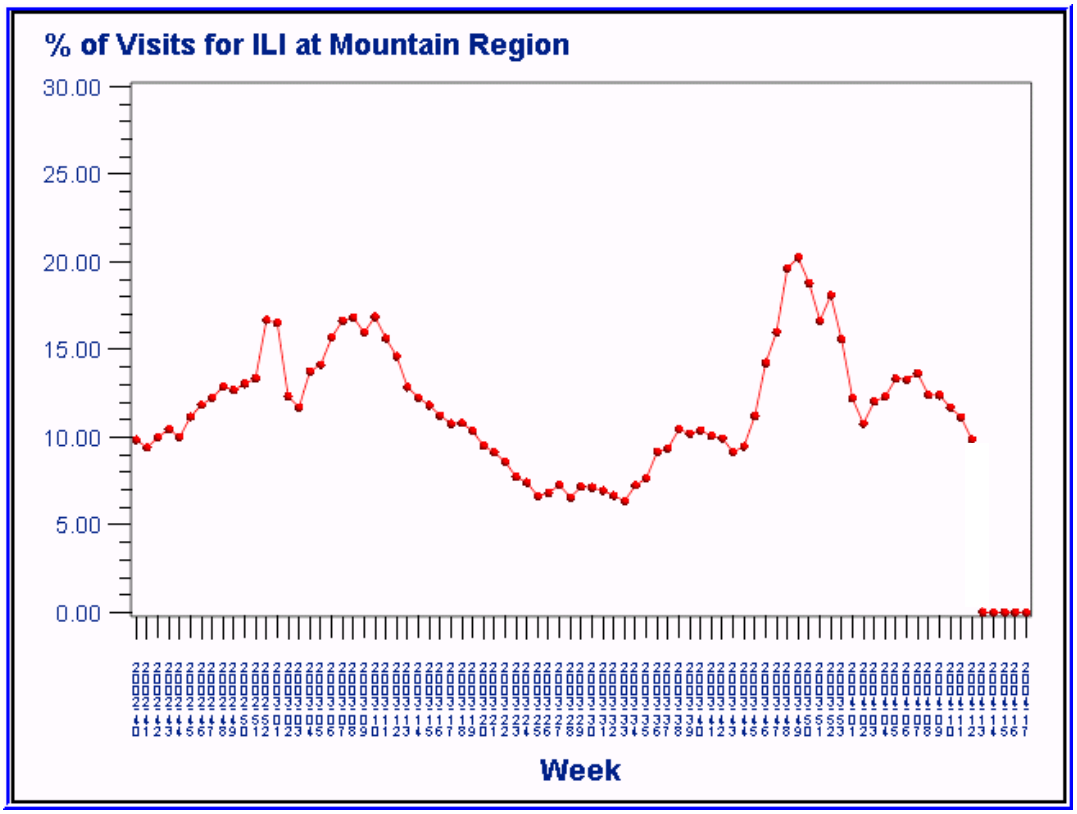

Figure 8: ILI ESSENCE graph for CDC Mountain regional grouping, week 12 2004

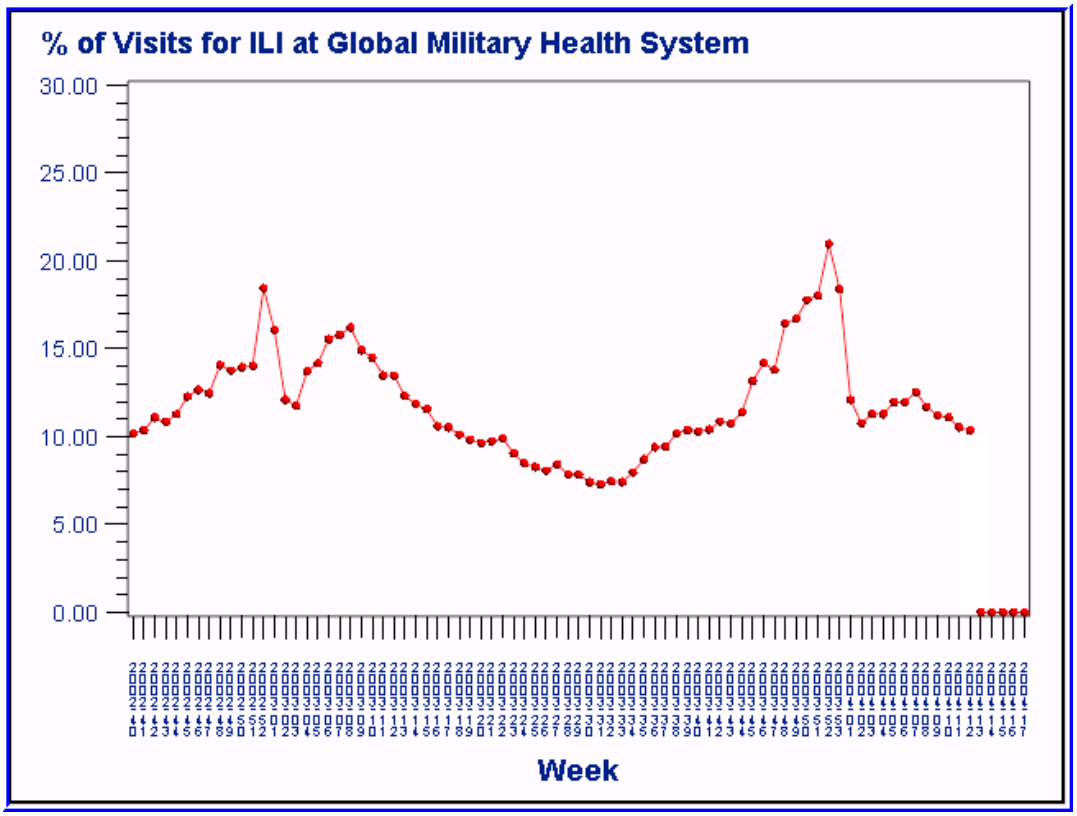

Figure 9: ILI ESSENCE graph for all DoD data, week 12 200

Currently ILI ESSENCE data are available to all operators with access through a secure website for ESSENCE. Instructions and documentation for ESSENCE and the ICD-9 set for ILI are available at DoD-GEIS public website www.geis.ha.osd.mil. The primary uses for this system currently include DoD's Global Influenza Surveillance weekly report and DoD SARS surveillance. The goal is improved and timely outbreak detection & monitoring for respiratory/flu-like illness in DoD, augmenting other systems. Places where expertise is developing in the use of this data are the Services epidemiology centers (Army Center for Health Promotion and Preventive Medicine (CHPPM), Navy Environmental Health Center (NEHC), Air Force Institute for Operational Health (AFIOH)), regionally (Army regional medical centers, Navy Environmental and Preventive Medicine Units (NEPMU's) and at research centers, especially the Naval Health Research Center (NHRC) in San Diego that partners with AFIOH in the influenza program.

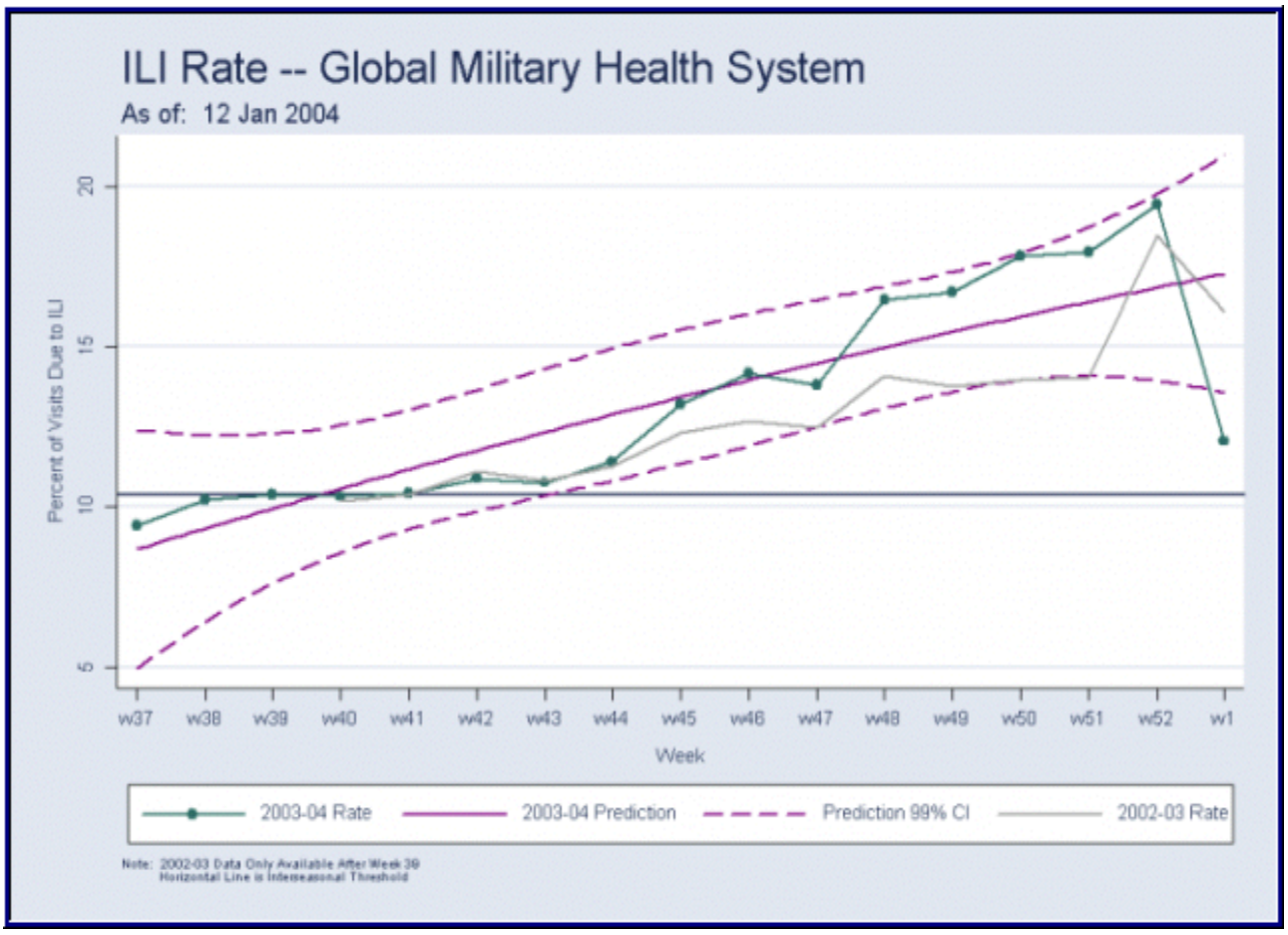

Figure 10: ILI graph from the weekly DoD Global Influenza Surveillance Program published report, AF Institute for Operational Health, Brooks City Base, San Antonio, Texas, week 1 2004

Figure 11 combines surveillance data from DoD and the CDC for comparison; DoD ILI activity (globally), DoD laboratory, CDC laboratory and ILI sentinel physician data are represented graphically. Traditionally percentage positive is used for influenza laboratory-based surveillance reporting as shown in the trend line in figure 4, right side Y-axis and that convention is used here but shown on the standard Y-axis in figure 11. Correlation between DoD and CDC data can be seen in the global and regional data for military populations and national data and for laboratory data as shown. Work is in progress to better quantify this correlation.

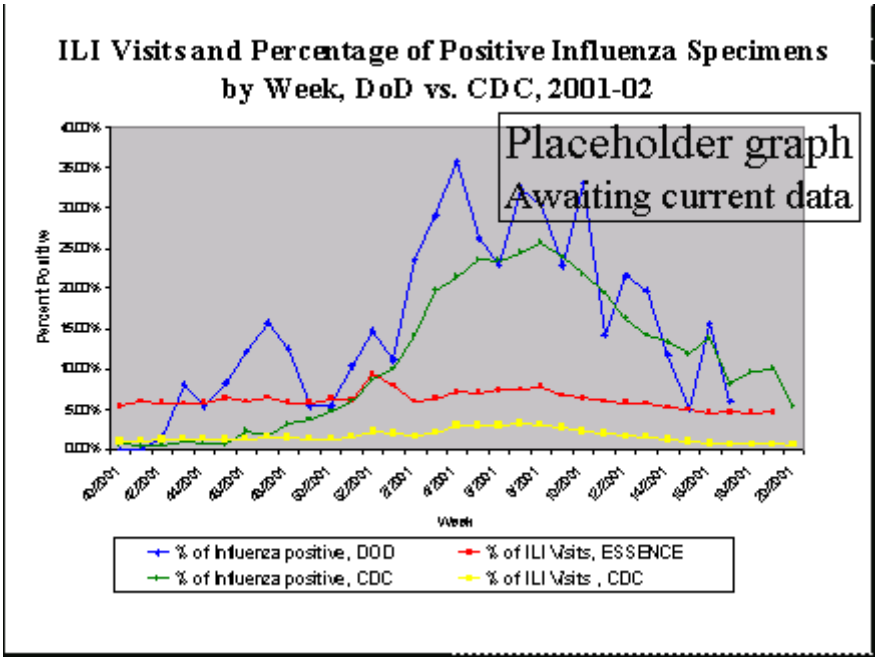

Figure 11: ILI data from DoD and CDC surveillance

There were unexpected benefits derived from the methods for ILI ESENCE surveillance when SARS surveillance needed to be quickly developed; because this study quickly resulted in an operational surveillance system just prior to WHO's SARS outbreak notification early in 2002, the collaborators in this study and GEIS were especially well positioned to use and integrate all partners' surveillance, including ILI ESSENCE, reporting and response capacity for this outbreak. System architecture, communication and reporting were already in place; vital links already existed in the influenza program between epidemiology and public health laboratory personnel who were already familiar with this project. ILI data baselines were already established when SARS concern arose. Timely DoD data suitable for respiratory disease outbreaks was logically organized and available for key leadership. Beyond this there is potential to use ILI data to facilitate targeted sampling for influenza strains or other respiratory pathogens and for prioritization of samples to improve surge capacity in epidemics or pandemics. Capability developed for SARS and influenza have implications for pandemic influenza planning and response as well.

5.0 CONCLUSIONS

Timely ILI surveillance is feasible with this methodology, as each patient becomes a sentinel using existing electronic data, ESSENCE infrastructure and global influenza surveillance collaboratively with GEIS. There is correlation of ILI activity in the military communities with CDC sentinel physician detected activity regionally and with influenza positive cultures. Useful comparisons can be made with national influenza surveillance system data. This surveillance capability is one component of a larger surveillance system that integrates and leverages existing respiratory disease surveillance through a unique linkage between syndromic and laboratory-based approaches. In concept it is analogous to the CDC's sentinel physician system but requires no special data collection from physicians and utilizes existing data sources, requiring little to no additional resources to operate. As part of influenza surveillance and ESSENCE this enhancement is

operational now, validation is underway and uses expand. An important DoD-wide SARS surveillance system followed immediately.

6.0 LIMITATIONS

ICD-9 code data is not the same as a medical diagnosis or a case definition; it is less specific. Although this technique has not yet been validated, work has begun on validation and the design facilitates this. No inpatient or mortality data is included and no medical encounters during deployment are captured. As yet only DoD medical encounters captured but the method is not limited to DoD. Finally there are some special problems generalizing from DoD populations. Some important factors to consider include: geographic, US & international, coverage is not homogeneous as military populations are clustered in some areas and not in others, demographics are different (e.g., age distribution, gender) and influenza vaccination rates are much higher for military than civilian populations. Although these limitations are important to remember, this technique and military population data can be used for DoD population surveillance and to augment other surveillance systems.

7.0 ACKNOWLEDGEMENTS

We would like to recognize these organizations and their personnel for their collaboration, achievements and contribution to this work.

- DoD-GEIS Central Hub, WRAIR, Silver Spring, MD
- ESSENCE group, Division of Preventive Medicine, Walter Reed Army Institute of Research, Silver Spring, MD
- Air Force Institute for Operational Health, Brooks City Base, San Antonio, TX
- Naval Health Research Center, San Diego, CA
- Office of the Assistant Secretary of Defense (Health Affairs), Washington, DC

8.0 REFERENCES

[1] RTO Reference Text – to be completed

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SYMPOSIA DISCUSSION - PAPER 10

Authors Name: LtCol MacIntosh (Col Cox speaker) (US)

Discussor's Name: Prof. Dr Fosse (NO)

Question:
How do you ensure the quality of the input to the global data base?

Author's Reply:
I am not aware of any system-wide validation, though work proceeds in this area at DoD GEIS-ESSENCE. There have been focused areas of validation at sites where there were confirmed outbreaks, including record review combined with evaluation of what appeared in the ESSENCE database. There is also comparison of ILI trends between ESSENCE and CDC data, as described in this paper. Finally, there are ongoing efforts on the clinical quality assurance side of the house to improve coding accuracy at MTFs across the DoD, including hiring more nosologists and making it simpler for providers to translate their diagnosis into ICD-9 codes.

Authors Name: LtCol MacIntosh (Col Cox speaker) (US)

Discussor's Name: Dr Nieuwenhuizen (NL)

Question:
The last slide read "no deployment data available". What is the role of the DOEHRS systems?

Author's reply:
DOEHRs system is garrison – related. There are, however, some data sources available for the field. In the future, TMIP (Threat Medical Information Program) will feed theater data, both health encounter and occupational/environmental, to CHCS-2, DOEHRS, and other systems.

Authors Name: LtCol MacIntosh (Col Cox speaker) (US)

Discussor's Name: Dr Reifman (US)

Question:
Influenza may not be a "good" illness to validate a syndromic system against, as influenza is part of the "normal " pattern of cyclic illnesses.

Author's Reply:
It is difficult to validate a syndromic surveillance system. Using a disease which is both common and has "gold standard" laboratory confirmation available is an advantage. We can also adjust the historical data to eliminate known outbreaks in an effort to better capture the "true" underlying baseline flu activity. We are also validating the system against other known outbreaks e.g., norovirus.

An Overview of the Medical Data Surveillance System

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ABSTRACT

In theater, it is essential that the services have a common medical system; therefore, a program was initiated to design, develop, and implement an automated system to process medical data on deployed personnel. Once it was demonstrated that medical information could be efficiently processed in theater, a research effort was undertaken to automatically analyze the data in order to detect deviations from historic illness patterns. Consequently, a system called the Medical Data Surveillance System (MDSS) was designed and developed as a Web enabled system for data analysis and reporting. The analysis capability of MDSS allows the user to compare the relative number of cases in one set of categories to those in another set across two different time intervals for a selected military unit. Or, the relative number of cases for two different military units can be compared during the same time interval. A unique set of signal detection algorithms called Dynamic Change-Point Detection (DCD) allows the user to select and analyze the entire population or those cases associated with a particular medical treatment facility (MTF), those cases associated with a particular Military Unit, or those cases associated with a group of Military Units that used a specified set of MTFs. Results are returned in a tabular format and as colored bar graphs. The analyses are conducted on the cases that have been reported, and on illness rates per thousand per day. Increasing and decreasing trends are identified in MDSS tables and graphs. The shifts and trends as well as bursts and outliers are labeled in the tables. Finally, MDSS generates a daily alert matrix for each MTF by automatically running the DCD algorithms for the illness and injury categories produced by MDSS.

1.0 INTRODUCTION

In the U.S. Navy, theater medical surveillance has its roots in a series of studies of illness among shipboard personnel that began in the late 1960s and continued through the 1970s. These studies were an effort to identify the factors related to the onset of illness among the crews deployed aboard U. S. Navy ships

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

[1-4]. Initially, it was necessary to collect the medical data by manually transcribing clinical information to data sheets, which were used to enter the data into an electronic database. As the shipboard studies continued, the manual data collection evolved into a semi-automated system where each patient encounter was documented on a form that could be optically scanned. Participating ships forwarded these sheets to the Naval Health Rsearch Center (NHRC) where they were scanned, entered into the database, and used to compile the Monthly Medical Services and Outpatient Morbidity Report for participating ships [5]. As automation of medical information in the field became more feasible, research efforts by NHRC led to the design and development of the Medical Data Tag for the capture and storage of medical information in theater [6]. The Field Medical Surveillance System was designed and developed by NHRC to assist with the diagnosis and tracking of illness incurred by personnel in field environments [7].

In theater, as elsewhere, it is essential that the different branches of the armed services have a common medical system. Therefore, the Office of the Secretary of Defense for Health Affairs (OSD [HA]) initiated the Theater Medical Information Program (TMIP) to design, develop, and implement an automated system to process medical data in theater. This effort began with each service determining its functional requirements. In the case of the Navy, SSC-SD was recognized as the Navy activity with the mission and necessary expertise for system engineering and development. Hence, SSC-SD was tasked to determine and document the naval theater medical information requirements. From the requirements that had been provided to OSD (HA), SSC-SD developed the Theater Medical Core Services (TMCS) as a prototype technology designed to meet many of the identified requirements. TMCS was able to capture selected clinical data, medical supply data, and staffing data; communicate that information up the chain of command; and use it to compile a variety of status reports.

Once it was demonstrated that medical information could be efficiently processed in theater, however, new questions appeared. How could one review all the reports that were submitted and be sure every anomaly was investigated? And, how can deviations from historic rates be detected as the data are being received? These questions were the subject of an independent research effort by J Pugh at SSC-SD. The culmination of this effort was a set of signal detection algorithms known as the Dynamic Change-point Detection (DCD) algorithms. The DCD algorithms provide the ability to evaluate new data upon receipt and:

- Rapidly set illness and injury baselines
- Set threshold values for various types of deviations from the baseline
- Flag changes from the baseline for a disease or disorder
- Determine when a change from the baseline began and ended

By systematically processing incoming data using the DCD algorithms, large quantities of data could be analyzed and deviations from the baseline could be flagged for investigation.

2.0 MDSS CAPABILITIES

To transition the array of surveillance tools to the Fleet, a research effort was initiated to create a system for medical surveillance of Navy and Marine Corps deployed forces. The primary objective of the system, called the Medical Data Surveillance System (MDSS) was to rapidly detect medical threats through the analysis of routinely collected patient data. Working as a team, personnel from NHRC contributed epidemiological expertise and technology developed through their field medical technologies program, and

SSC-SD personnel contributed statistical expertise and technologies developed through their intelligence, surveillance, and reconnaissance program. The team recognized that the key to conducting an effective medical surveillance program is to be able to rapidly aggregate and process data from a wide range of sources and then quickly disseminate the results. Thus, MDSS is a Web-enabled system. It is designed to accept data from the U.S. Navy's Shipboard Non-tactical ADP (SNAP) Automated Medical System (SAMS) and from the U.S. Military's Composite Health Care System's Ambulatory Record System. As data are acquired by MDSS, they are aggregated in several ways. These data are used to form the Disease and Non-Battle Injury (DNBI) reporting categories, categories for ill-defined conditions, Chemical-Biological-Radiological (CBR) groupings, key symptom categories, and the major categories of the International Classification of Diseases, Ninth Revision (ICD-9) currently used to document diagnoses in U.S. military medical records. The DNBI categories support the standard monitoring of deployed forces, the CBR groupings are designed to highlight effects of a CBR exposure, and the ill-defined condition and key symptom categories allow separate symptoms such as fever or diarrhea, to be trended and analyzed from data derived entirely from diagnostic codes.

After the data are received and stored, they can be used to automatically generate the DNBI report that summarizes DNBI incidence counts, incidence rates, lost work days, days of light duty and numbers of outpatients and inpatients. Similar reports are generated for ill-defined conditions, the CBR categories, key symptoms, major ICD-9 categories and reportable conditions. In addition, information needed for the Medical Event Report is produced for any patient with a condition included on the Tri-Service Medical Event List. A list of the specific individuals who contribute to the counts on these reports can be readily determined by clicking on the category of interest. Moreover, detailed information on any one of these individuals can be obtained by clicking on the person's name. Users can also create Ad Hoc reports by downloading the patient data into an Excel spreadsheet. Then using the Excel Wizard, the user can create tables and graphical displays of the information.

The analysis capability of MDSS includes DCD and 3 non-parametric statistics. The non-parametric statistics were imported from the Enhanced Consequence Management Planning and Support System developed with support from the Defense Advanced Research Projects Agency. The non-parametric functions allow the user to compare the relative number of cases in one set of categories to those in another set, across two different time intervals for a selected military unit. Or, the relative number of cases for two different military units can be compared during the same time interval. The DCD capability allows the user to select for analysis the entire population; or, the subset of cases associated with a particular medical treatment facility (MTF), those cases associated with a particular Military Unit, or those cases associated with a group of Military Units that used a specified set of MTFs. Then the user specifies the time period of interest and the illness category of interest. Results are returned in a tabular format and as colored bar graphs. The analyses are conducted on the number of cases that have been reported, and on illness rates per thousand per day if Population At Risk information has been provided to the system. Increasing and decreasing trends are identified in MDSS tables and graphs. The shifts and trends as well as burst and outliers are labeled in the tables. On colored graphical displays green is used for days within normal limits, yellow is used for days that show some increase from the background rate, and red is used to flag those days that are two standard deviations above the baseline, or when a trend or shift is statistically significant. Black is used for days that are three or more standard deviations above the baseline. Finally, the beginning point and ending point of trends are indicated when the color changes to or from green.

Because there is a difference in the operation of MTFs on weekends and holidays, there is a dramatic difference in the number of patients seen. MDSS adjusts for this phenomenon by developing and using separate baseline and threshold values for those days. Finally, MDSS generates a daily alert matrix for each MTF by automatically running the DCD algorithms for the illness and injury categories produced by MDSS,

and it displays the results for each of the past 10 days. As a result, at the beginning of each day the user can view the status of each illness and injury category and see if there is any single day that differs from the others, and see if there are any temporal patterns in the recent past.

3.0 THE DCD METHOD

In this section the development of the DCD is discussed to help provide an understanding of this analytic technique and its value for medical surveillance. The approach starts with viewing medical surveillance as being analogous to a manufacturing problem where a production line is monitored for defective parts. From this perspective, an increase in the rate of defects would indicate the manufacturing process has been degraded. In an analogous manner, an increase in patients would indicate that the health protection process has been degraded. Further, in the development of DCD a distinction was made between two types of changes. One is a sharp change, a stark departure from the historic levels that often, just as rapidly, returns to the norm. This is known as a burst or outlier. The second type of change is generally a smaller deviation from historic levels but is sustained over an extended period of time. This type of change is known as a shift or trend. The first change may reflect a break in the process, while the second may reflect the need for an adjustment in the process or recalibration.

The recognized method for detecting bursts and outliers is the Shewhart test, and when detecting shifts and trends the CUSUM technique is used [8]. For the medical application, however, the CUSUM needed some extensions. First, once a change is detected, it is important to know when the change began and when it ended. Knowing the day that an increase in patient visits started can be useful in determining the cause of the change. Also, it is necessary to know if the number of cases decreased following the implementation of an intervention, when assessing the efficacy of the intervention.

In addition to establishing start and stop points, the CUSUM needed to be extended to small samples. Specifically, the need was to extend the CUSUM statistic so that that stable baseline and threshold values could be established in a few days rather than requiring 1 or 2 months of data before these parameters could be established. This is particularly important when deploying to a new area where no information is available on disease rates. This capability is also valuable when conducting Ad Hoc analyses because various subgroups can be readily investigated with respect to their unique historic values. The desired extensions to the CUSUM technique were derived by starting with the Neiman-Pearson fundamental theorem of statistics [9], thereby, generating the uniformly most powerful test. The resulting test was combined with the Shewhart test to form the DCD algorithms.

The advantage that DCD had over other methods was determined by conducting a Monte Carlo study where a series of random data sets were generated. Various types of signals (slopes, single points, square waves, saw tooth.) were prepared and embedded in random data (i.e., noise). Then, DCD and each of the alternative methods, including regression tests, moving average techniques and F-ratios, were used to find the signal within the noise. In each case the threshold was set so that random deviations exceeded the threshold 5% of the time. The power of each method was assessed by the frequency that the embedded signal was detected by the test and how long the signal was present before it was detected. In the case of DCD, separate thresholds were set for the Shewhart and CUSUM statistics. The results showed, if the signal was present during the entire period of observation, DCD was as good or better than any other method. However, if the signal began and/or ended one or more times during the interval, the DCD algorithms were superior to all other methods for all types of signals.

4.0 EXERCISES AND IMPLEMENTATIONS

Although various surveillance components have been demonstrated and tested over a period of years, MDSS as an integrated system was first exercised in Cobra Gold 2001. In that exercise it demonstrated immediate utility. Data were captured using SAMS and were supplied to MDSS every 15 minutes. In less than one week a baseline value and threshold were established from the incoming data. During the exercise unusual occurrences of dermatological problems was flagged by the system. Using the patient information report, the personnel located at the Joint Task Force Headquarters were able to determine that many of the cases were occurring within a particular unit. A team was sent to investigate and they found that the problem was caused by mite bites. It was found that because many of the personnel from the affected unit had not sprayed their mosquito netting, mites were getting through. Once the netting was treated properly the problem subsided. Because of the rapid detection, the ability to identify the population affected, and the intervention taken, the elevation in dermatological problems lasted just 2 days. As a result of this experience the patient drill-down feature was added to MDSS so that the individuals contributing to a particular total can be found simply by clicking on the display.

MDSS was also exercised in Kernel Blitz during 2001. On the one hand, users found that the system was useful for processing the quantities of data that were received. By using MDSS, environmental health personnel could ensure all deviations from baseline values would be flagged for investigation. On the other hand, a need was identified for an alerting mechanism that would prompt a person to run an analysis on the data being received. Consequently, the alert matrix was implemented so that a standard set of DCD analyses would be performed each day and displayed, thereby, providing an automatic alert.

Early in 2001 the US CINCPAC Surgeon recognized the need for a medical surveillance system in the Korean Theater of Operations (KTO). Therefore, a Theater Health Assistance Team was sent to South Korea to assess the potential of new and innovative technologies for enhancing health surveillance. As a result, a plan for developing a system that built upon the extant infrastructure was devised. Basically, the concept was to feed MDSS with the Standard Ambulatory Record Data generated by the CHCS Ambulatory Record System. An initial capability was developed and tested at the Naval Medical Center, San Diego. Then, in September 2001 a team implemented the system at the 121st evacuation hospital in Korea. Soon, it was found that the difference in hospital operations during weekends and holidays created an artifact that interfered with data analysis and interpretation. This problem was resolved by separating full staff days from reduced staff days and analyzing the data sets separately so that each one is evaluated with respect to its own baseline and threshold.

Currently, MDSS is being used routinely in KTO for medical surveillance and, in September 2003, MDSS was formally transitioned to the Theater Medical Information Program. The technology described in this paper is the subject of one or more pending patent applications and is available for licensing from the U.S. Navy. Licensing inquiries may be direct to Ms. Jamie Pugh.

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Challenges of Electronic Medical Surveillance Systems

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ABSTRACT

In this paper, we discuss the technical challenges of electronic medical syndromic surveillance systems intended to provide early warning of bioterrorist attacks and naturally occurring epidemics. The discussion includes challenges associated with both civilian and military environments. In particular, we address the challenges in: (1) establishing an automated data collection infrastructure, (2) achieving timely access to quality data from disparate sources, (3) developing sensitive and specific outbreak detection algorithms, and (4) developing comprehensive and realistic simulation models for detection-algorithm development and validation. In addition, we identify unique attributes of military and North Atlantic Treaty Organization settings that may affect the development, deployment, and usage of medical surveillance systems. We conclude that considerable work and research are needed to overcome these challenges, that the information provided by these systems may lack the necessary specificity for follow-on mitigating actions, and that their cost-effectiveness and practical relevance, vis-à-vis the traditional reliance on health care providers to identify outbreaks, is still to be demonstrated.

1.0 INTRODUCTION

The 2001 anthrax attacks in the U.S. and the international outbreak of Severe Acute Respiratory Syndrome (SARS) heightened the importance of information technologies that could provide early warning of bioterrorist attacks and naturally occurring epidemics. Electronic medical surveillance systems, whose genesis precedes these recent events and which are oftentimes referred to as syndromic surveillance systems, are being widely investigated as a potential dual-use early indicator of abnormal events. These systems are not intended for disease diagnosis or longitudinal health monitoring, but rather to detect impending epidemic outbreaks and identify infected individuals early in the course of their disease through disparate data sources before a confirmed diagnosis is made. Generally, these syndromic systems target the detection of abnormal patterns in non-specific data, such as school and work absenteeism, over-the-counter pharmacy sales, nurse triage calls, and data logs of clinical symptoms and signs (in the form of acute respiratory infection or gastrointestinal illness) from encounters with primary care physicians. More recently, there has been an interest in broadening the scope of such systems to improve their timeliness by integrating public health information with risk indication and vulnerability information.

A multitude of electronic medical syndromic surveillance systems are being investigated for both military and civilian settings [1,2]. The various services and agencies of the U.S. Department of Defense (DoD) are sponsoring the development of different systems for both deployed and garrison-based forces, and many other

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government agencies as well as just about every state and local government in the U.S. is investigating their own approach. While there is clearly no lack of interest and resources being targeted to the development of syndromic surveillance systems, before their potential benefits can be fully exploited—not to mention the questionable practical usefulness of these systems' outputs—a number of key technical challenges need to be addressed and overcome.

In this paper, we identify and discuss numerous technical challenges for developing and deploying medical surveillance systems in both military and civilian settings. In particular, we address the challenges in: (1) establishing an automated data collection infrastructure, (2) achieving timely access to quality data from disparate sources, (3) developing sensitive and specific outbreak detection algorithms, and (4) developing comprehensive and realistic simulation models to generate development and validation data for the detection algorithms. Moreover, we discuss the unique attributes of military and North Atlantic Treaty Organization (NATO) settings that may affect the development, deployment, and usage of these systems.

2.0 DATA ACQUISTION INFRASTRUCTURE

One of the major challenges in implementing any type of automated medical syndromic surveillance system is establishing and maintaining the information processing infrastructure to collect, store, transmit, and share data for analysis. This is especially true in a mixed military/civilian environment and in locations where military forces have been forward deployed. Military and civilian agencies use disparate information systems and data communications networks to record, store, transmit, and consolidate data relevant to epidemic surveillance. Military and civilian medical information systems are often incompatible in hardware, software, data architecture, and/or data transmission protocols. There are even significant incompatibilities among government agencies and within the same agency, the individual military services, organizations, and functional activities. Some of the most useful data are not even collected digitally or are so sensitive (e.g., intelligence data) that merging them with less sensitive private data (e.g., nurse triage, doctor visits, pharmaceutical sales, school/work absenteeism) is difficult, if not impossible.

Within the DoD Health System, a 20-year effort has been underway to create a comprehensive "cradle to grave" global health information system that captures, stores, processes, and facilitates analysis of the medical records of military members, retirees, and their families. The effort is culminating in the implementation and population of a world-wide DoD clinical data repository that takes inputs from disparate DoD medical and non-medical information systems to consolidate various types of data from outpatient and hospitalization records from military and military contracted medical facilities, including clinical symptoms and signs, chief complaints, test and laboratory orders, and pharmacy data. Only recently, through initial implementation of its Theater Medical Information Program (TMIP), has the DoD effort encompassed the forward deployed military forces in war zones like Bosnia, Kosovo, Afghanistan and Iraq. Along the way, this effort has encountered significant bandwidth and information processing and infrastructure roadblocks. Fortunately, some major innovative solutions in military information processing strategy are nearing implementation. Figure 1 provides an overall illustration of the various sources and types of data that need to be integrated.

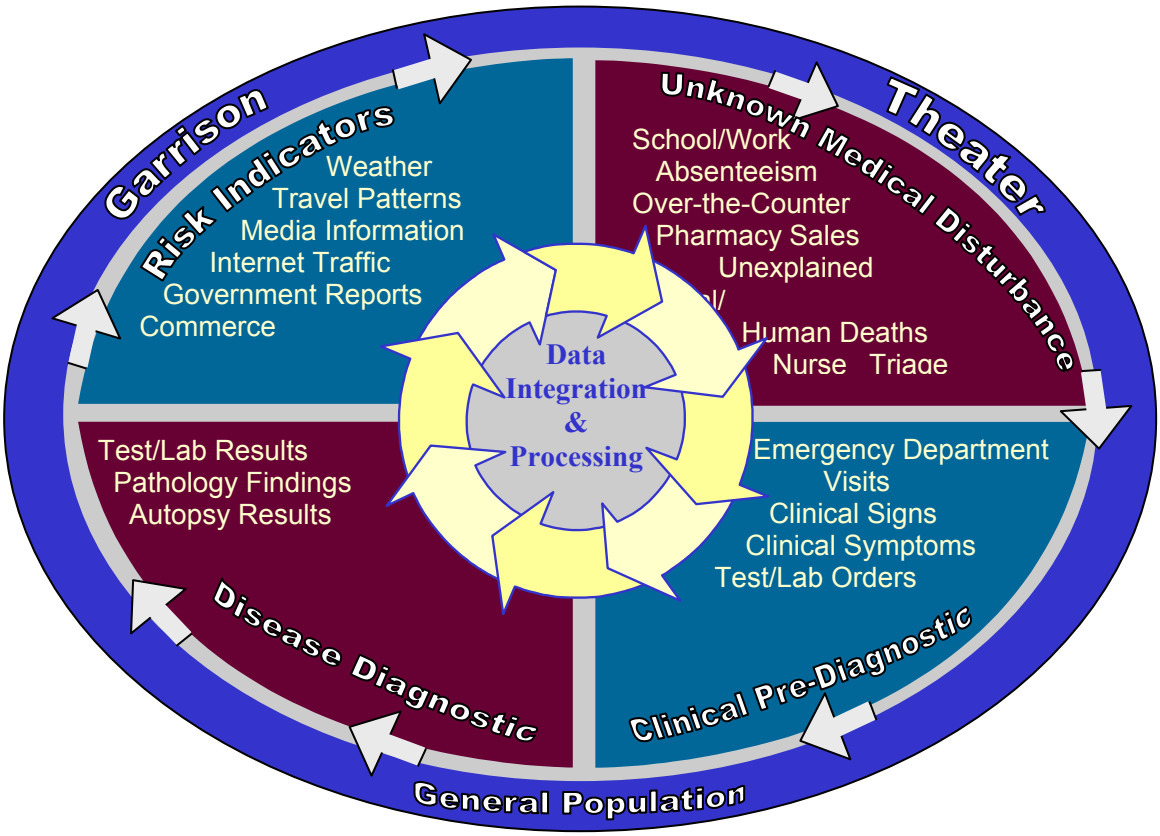

Figure 1: Sources and Types of Medical Syndromic Data that Need to Be Collected, Transmitted, Stored, and Processed for Analysis.

An emerging information architecture doctrine for the DoD, called Global Information Grid (GIG) [3], was born from concerns regarding interoperability and end-to-end integration of automated information systems. In this architecture, a distributed network-centered information architecture serves as both a collection center for information from those generating it as well as a source of information for those needing it, without a pre-planned or required communications link between information generators and information users. The emerging "net-centric" architecture for military medical operations [4] is based on a related concept (see Figure 2). Within net-centric operations, many of the medical information deposits will be of the "transmit and forget" nature (those inputting the information are not concerned with who will use it), while many of the information queries will be "blind" (those requesting and using the information will not be aware of its source). In order for a world-wide (or even regional) syndromic surveillance network to be efficiently and securely implemented within the military and between the military and civilian digital information networks that would serve as data collection sources, a net-centric GIG-type architecture is required.

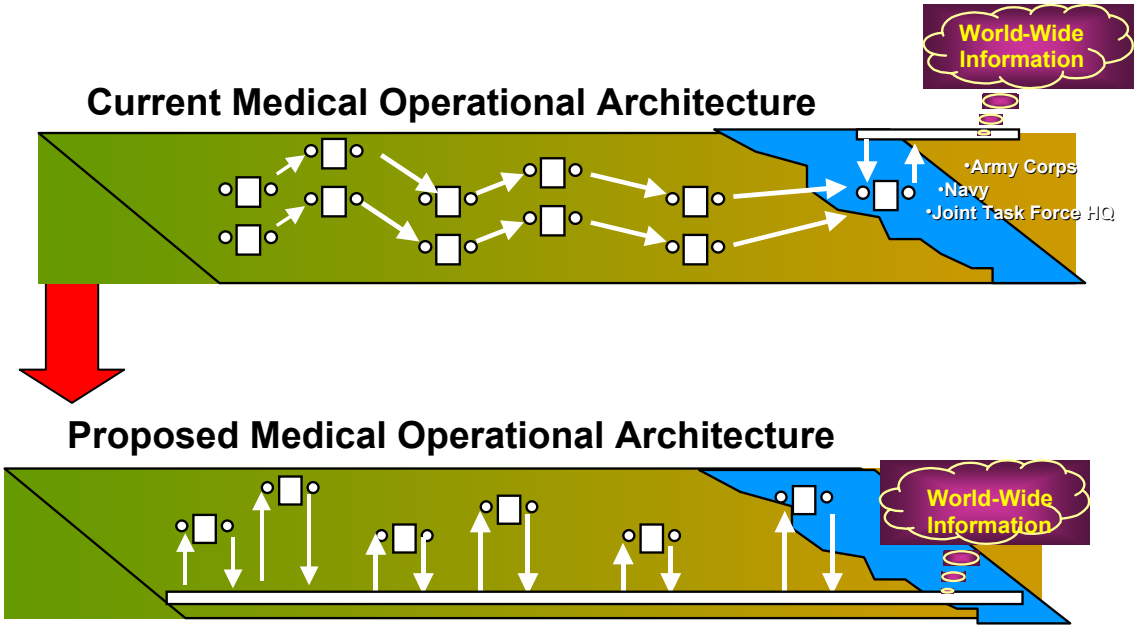

Figure 2: Proposed Network-Centric Medical Information Operational Architecture

Among NATO military members, several biosurveillance, medical information architecture standardization and systems integration and interoperability efforts are underway. This is occurring under the auspices of the Medical Information Management Systems Working Group, sponsored by the Committee of the Chiefs of Military Medical Services in NATO (COMEDS), and the Nuclear/Biological/Chemical (NBC) Medical Working Group of the NATO Agency for Standardisation. Full integration of health-care data from NATO-member government agencies, their military health services, and private health provider counterparts sufficient for efficient syndromic surveillance is a long-way off. To accelerate this process, one potential solution is the use of an "open systems" data communications network and/or open systems network enabling software that resides at the transport and network layers underneath the individual applications, session, and presentation layers, but on top of hardware device data-link and physical layers according to the Institute of Electrical and Electronic Engineers data transmission hierarchy. A standard "open systems" data architecture with standard data dictionaries, data formats, and data compression algorithms is also required if data are to be shared. Adopting open systems is key to facilitating interoperable data communications and information sharing. It is also the only economical way to deliver a solution that measurably solves the problem [5].

Sponsored by numerous U.S. government agencies, academic institutions, information technology firms, and private health care organizations and insurers, the National Forum for Health Care Quality Measurement and Reporting [6] initiated a massive project to identify a strategy and an agenda for establishing the type of national health information infrastructure that is essential to an effective medical syndromic surveillance data collection and distribution system. Initially, the Forum commissioned background papers and convened top leaders in healthcare and information technology to: 1) characterize the current state of the nation's health information infrastructure, 2) identify the primary impediments to achieving the timely flow of necessary health information across the continuum of care, along with ways to eliminate these barriers, and 3) identify actions needed to create the political will to adopt the laws, standards, business practices, and technologies necessary to create a state-of-the-art national health information infrastructure. Without such a massive effort,

the obstacles to the comprehensive near real-time health information processing required for effective syndromic surveillance may be insurmountable.

3.0 DATA SOURCES AND TIMELINESS

Selecting data sources for inclusion in an electronic medical surveillance system will be partially dictated by which phase of the detection timeline is being targeted. Figure 3 illustrates how this might be conceptualized. The far left of the spectrum represents the most timely but least specific stage along the timeline, and affords the greatest lead-time for preparation and response to an impending bio-event. The far right represents the most specific stage, the time at which a definitive diagnosis is made within a certain community. In between these two extremes are the two phases of the timeline at which most medical syndromic surveillance strategies are directed.

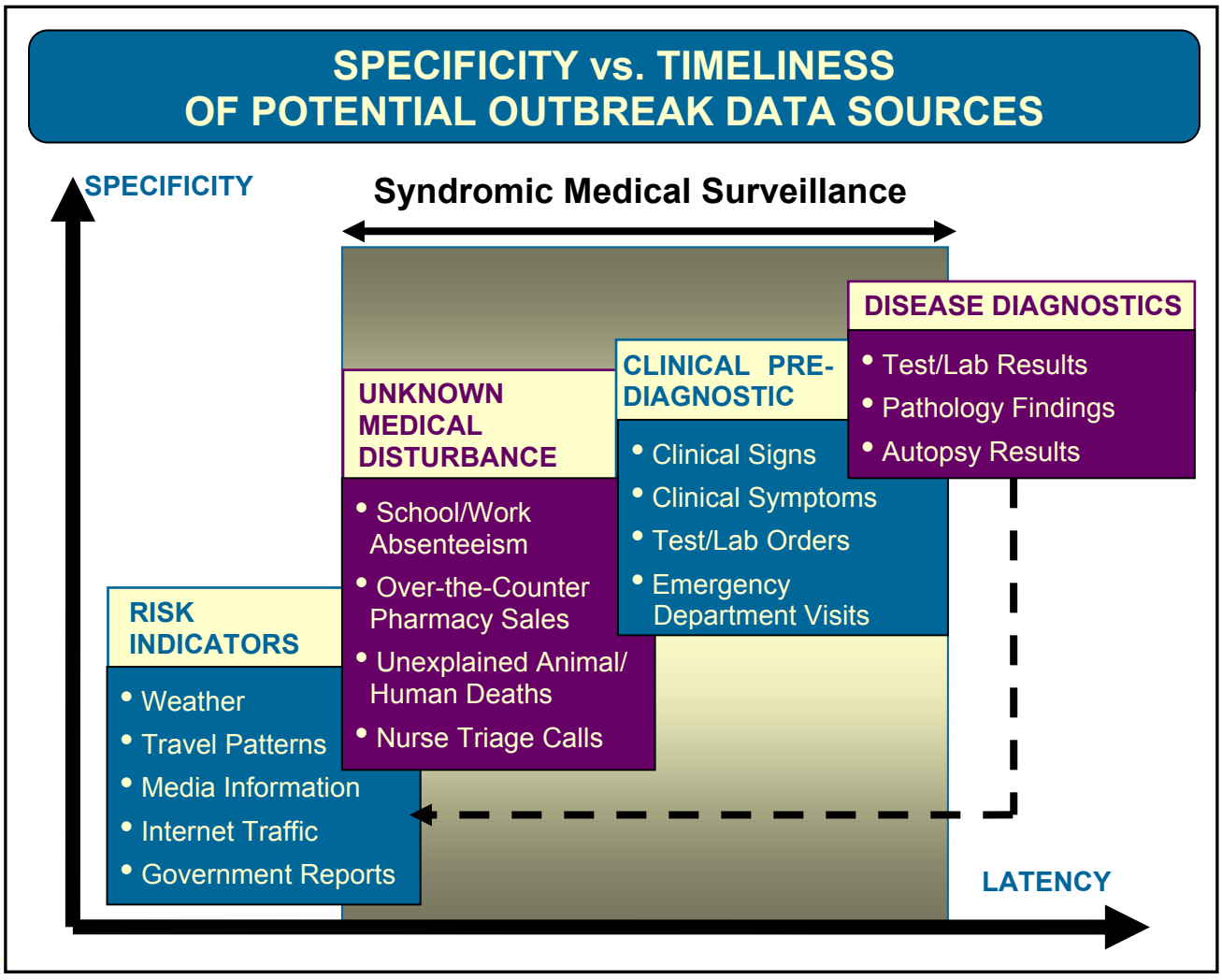

Figure 3: Conceptual Representation of Specificity versus Timeliness of Potential Data Sources for Electronic Medical Syndromic Systems

Focusing on the far left of the spectrum, the "first alerts" available may consist of risk indicators that a community is at risk for a bio-event, i.e., "conditions are favorable," prior to an actual epidemic event in that community. These markers of potential vulnerability may in some cases be extracted from data sources not directly related to healthcare. For example, local enviroclimatic information may indicate conditions are appropriate to support transmission of an insect-vectored pathogen. In the event of a known or suspected infectious disease outbreak overseas, inspection of air travel patterns might allow a reasonable prediction of which U.S. cities would first be affected due to translocation of a pathogen. An example is provided by the West Nile virus outbreak in 1999, suspected to have translocated from Israel to the U.S. [7]. Given that the overwhelming majority of air travel from Israel to the U.S. routes through the JFK airport, it may not be surprising that New York City was the initial U.S. site for ecological establishment. As noted in the SARS epidemic, information issued by foreign government health agencies may not accurately reflect the full scope and severity of an outbreak. While potentially anecdotal and non-specific, data sources such as media reports, internet traffic, and telecommunication patterns may reflect the level of anxiety within an affected community and provide clues about the true impact and extent of the event. In addition, commerce information about livestock restrictions or factory closings may also suggest a greater level of concern than that conveyed by an official government body. These indicators described above could potentially be used to assess risk for a given community prior to an actual outbreak in that community.

Moving further to the right on the timeline, unknown epidemic outbreak detection could be targeted. A bio-event could be occurring within a community, although little is known about its identity. At this stage, indirect and non-specific medical indices might be useful. Potential markers to be analyzed include such things as rates of school and work absenteeism, over-the-counter pharmacy sales, and grocery sales. If available prior to a spike in healthcare visits, such markers might be useful in the "pre-clinical" setting, as discussed by Buckeridge et al. [8]. However, the timing of such markers relative to clinical presentation has been inconsistent, suggesting this relationship is more complex, and may vary depending upon the specific pathogen and/or environment in which the epidemic occurs. For example, Musen et al. [9] noted that, during a cryptosporidiosis outbreak in Milwaukee, Wisconsin, in 1993, school absenteeism peaked four days after the peak in emergency room visits occurred, contradicting conventional notion (and Figure 3) that school absenteeism peaks before emergency department visits [8]. Gross clinical markers, such as calls to nurse triage lines and an increase in unexplained human or animal deaths, might also be informative.

The next major stage along the detection timeline would be expected, in most cases, to closely follow or be nearly coincidental with the last stage. A medical condition with more defined characteristics is detected. Markers would still be primarily pre-diagnostic where direct clinical information, predominantly in the form of emergency department visits and associated patient symptoms and signs, is exploited. Syndromic surveillance based on defined categories of illness with certain clinical characteristics, such as gastrointestinal or respiratory distress, targets this portion of the timeline. Examples include syndromic surveillance systems such as the Real-time Outbreak and Disease Surveillance (RODS) and the DoD Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE) [2,10], among many others. Military disease and non-battle injury (DNBI) categories represent another similar, but less specific, way to classify medical events [11]. An illness is categorized broadly into a review of systems-type category, such as gastrointestinal or dermatologic, without qualifying characteristics or an intention to capture certain diagnoses.

The stage at the far right of the spectrum represents specific disease surveillance based on diagnostic information. While such information provides little or no response lead-time for the region from which the data originates, it is very specific and of great potential value to areas geographically remote. The performance and interpretation of diagnostic tests, however, are themselves sometimes subject to debate. For

example, the U.S. Centers for Disease Control and Prevention and the DoD do not routinely accept each other's results from environmental biosensors due to a lack of standardization between the two groups [12]. Although these sensors are not diagnostic clinical assays, the results from these sensors form the basis for recommendations regarding follow-up testing, which may impact decisions related to public health and clinical care. As described above and depicted by the dashed line in Figure 3, the presence of an epidemic in one community may prompt an analysis of risk potential elsewhere. One community's (or one segment of a population's) diagnostic information can serve as another's early indication and warning. For example, through the use of cross-correlation analysis of different time series, Sebastiani et al. [13] have shown that cases of respiratory syndromes in a pediatric emergency department can predict influenza morbidity and mortality in the general population up to three weeks in advance.

Regardless of the type of data sources included, several shared challenges to their utilization are apparent. Accuracy/validity of data, reliability/reproducibility of assessments or measurements necessary for data acquisition, accessibility of data, and protection of patient privacy are some of the key issues that must be considered in any surveillance system [2,10]. Lack of standardization in data entry and format, such as the use of free-text chief complaints from emergency departments versus the International Classification of Diseases, 9th Edition, (ICD-9) coding, as well as non-uniformity in definition of disease clusters are additional challenges currently being addressed by developers of syndromic surveillance systems. Motivated by concerns that ICD-9 codes are too fine-grained for bioterrorism detection as well as potential coding inaccuracies and inter-coder variability, several systems employ clustering of ICD-9 codes to define syndromes of interest [13]. Automated data collection within any electronic surveillance strategy is optimal, obviating the need for duplicative data entry into a system separate from that for which the data were collected. Logical data filtering and normalization are also key requirements, as integrated data are generally not readily suitable for traditional statistical analysis and may represent terabytes of information that require appropriate presentation to an analyst in order to balance the need for sensitivity with a low false positive rate.

It is important to note that the availability of accurate and reliable data is critical not only for the actual implementation and deployment of near real-time electronic medical syndromic detection systems, but also plays a key role in the development and validation of such systems.

4.0 OUTBREAK DETECTION METHODS

While a comprehensive and robust data acquisition infrastructure provides the necessary backbone for near real-time access to disparate data sources, the core of electronic medical surveillance systems lies within the data analysis algorithm. The vast majority of the proposed algorithms correctly concentrate on outbreak detection as opposed to outbreak diagnosis, as the latter is event specific, and as such, cannot identify an unmodeled, unanticipated outbreak. Outbreaks are detected as deviations between historical data representing "baseline" or "expected" values and current observed data to determine, for example, if patients are exhibiting unusual symptoms for the time of year, geography, and population. Baseline or expected values, determined for different data sources employing different mathematical formalisms, must reflect spatial variations and temporal periodicities (both daily and annual), such as increases of emergency department visits over the weekends when primary care offices are closed and during allergy season and winter months [15]. This recurrent (usual) incidence of cyclic diseases should be part of the "normal" pattern of diseases in computing the expected values, in order to recognize unusual patterns when compared with the usual.

Data-driven statistical detection approaches involving temporal, spatial, and spatio-temporal analysis have been proposed [10]. Temporal analysis accounts for the time progression of the disease/attack outbreak in a

fixed geographic location; spatial analysis examines the spatial distribution of observed cases in contrast with the background distribution for a fixed time or time interval; and spatio-temporal detection approaches consider deviations in both time and space. Knowledge-based methods that integrate surveillance data and knowledge have also been proposed, but those, perhaps due to their more intricate conceptualization and difficulty automating, are not as advanced as purely statistical methods [8]. The potential advantage of this approach is that in contrast with statistical methods that operate on low-level data, knowledge-based methods operate on data at a higher level of abstraction akin to human reasoning and could potentially represent more complex relationships, such as the modes and rates of infection transmission.

The development of systems for near real-time detection of infection outbreaks based on geographic information, both spatial and spatio-temporal approaches, faces considerable hurdles. In addition to the usual non-availability of the necessary data, for example, hospital information systems generally contain no information regarding a patient's work or school location, the information extracted from those data may be ambiguous, geographically diluted, and lack specificity. For instance, people may live, work, attend school, purchase medication, and seek medical attention in geographically dispersed locations, and exposure may occur elsewhere. Moreover, changes of demographics in a given location, e.g., construction of a large nursing home, can cause nonlinear changes in the baseline data for that location and potentially eliminate that location from analysis until updated representative baseline data can be re-established. Some of these issues, however, may not be a factor in military settings where residence, work, school, pharmacy, and health care may occur in the same restricted geographical area.

Another important consideration in the development of spatial and spatio-temporal approaches is the partitioning of a given area into a number of regions or clusters in which analysis is performed. Except for the limits imposed by the aggregation level for which data are available, predefined geographical boundaries should be avoided, as they can affect the degree to which spatial events can be detected. A desirable approach is to let the algorithm identify cluster boundaries "on-the-fly" by combining any number of close locations into the same cluster so that the most likely suspect cluster, that is, the one in which there is maximum mismatch between observed data and predicted data, is detected [16].

Due to data accessibility, lack of information ambiguity, and long experience and availability of time-series analysis methods, most detection approaches rely on temporal data analysis. A wide variety of methods exist to predict the expected value of time-series data. The simplest method, such as those based on control charts, base their predictions solely on historical data. In this approach, the expected data are simply a theoretical mean over time, which is constant for that time interval (day, week, or month of the year), and an outbreak is detected when the deviation between the expected and observed data is larger than a pre-specified threshold, typically some multiple of the standard error of the sample mean [10]. One of the limitations of such fixed seasonal models is that their estimate is constant and does not account for recent trends in the data.

More sophisticated approaches, such as regression models and classical autoregressive moving average (ARIMA) models that make estimates based on both historical data and current data, have also been proposed [10,16,17]. In this approach, outbreak detection involves comparing observed patterns with those predicted by a mathematical model. The primary benefit of ARIMA models is their ability to correct for local trends in the data so that what happened on previous days is incorporated into today's prediction. As discussed by Reis and Mandl [15], the incorporation of local information works well, for example, during a particularly severe flu season, where prolonged periods of high visit rates are adjusted to by the ARIMA model, thus reducing the occurrence of false alerts. However, in a slowly spreading outbreak, model corrections to local variations can cause ARIMA models to "adjust" to the actual outbreak, leading to over predictions and missed detection. A potential solution is to employ a hybrid detection system, incorporating both a fixed seasonal models and ARIMA models [15].

A common issue of these methods is the tuning of the algorithms to increase detection sensitivity (i.e., to decrease the possibility of missing the detection of a true event) on the first few days after the onset of an outbreak. The challenge is due to the considerable amount of daily fluctuations or signal noise in the data and the trade-off between detection sensitivity and specificity. Signal noise impacts model accuracy, leading to prediction errors, which in turn cause miss detections and false detections. Miss detection occurs when noise in the model's predictions masks the effects of actual outbreaks, lowering the system's overall sensitivity. False detection occurs when noise spikes in the model's predictions are detected as possible outbreaks, lowering overall specificity [17]. Invariably, tuning an algorithm to improve detection sensitivity deteriorates detection specificity and vice-versa.

Tuning an outbreak detection algorithm to increase sensitivity at the early stages of an outbreak can be theoretically achieved by defining the appropriate alert thresholds and using multi-day temporal filters, in which a weighted prediction over multiple days is aggregated and compared to a threshold [17]. However, this selection is not an easy one, as the optimal threshold levels and filter weights depend on the magnitude and temporal evolution of the outbreak. Without knowing the nature of the outbreak in advance, which we never do, it is not possible to optimize these parameters. For example, to detect an outbreak in the noisiest environment, that is, to detect a small and slowly evolving outbreak, the greatest sensitivity is achieved by using a uniformly weighted multi-day temporal filter capable of smoothing out noise and picking up weak signals over many days. In contrast, to detect a large and fast evolving outbreak with low noise-to-signal ratios, the greatest sensitivity is achieved by using an exponentially weighted filter, which performs the least smoothing as it places the heaviest emphasis on the most recent days. However, this also means that less weight is attributed to the wider temporal context, decreasing the system's capability to detect small signals over many days, and limiting the ultimate sensitivity that the system can reach. As Reis et al. have demonstrated [17], different threshold and multi-day filtering strategies offer superior sensitivity for a particular stage of the outbreak. Because we never really know at what stage of the outbreak we are at any given time, it is not possible to optimize such selections. Furthermore, they have also shown that, regardless of the weighting scheme, detection sensitivity at the first stages of an outbreak drops significantly when the magnitude of the simulated outbreak is within one standard deviation of the historical mean. Hence, even if optimally tuned, it is unlikely that these systems could systematically pick early outbreaks if their magnitude is small.

5.0 OUTBREAK SIMULATION TECHNIQUES

An important step in the development of computer-based surveillance systems is validation. System validation allows for the determination of which outbreaks can be detected, how large they must be to be detected, and how early they can be detected. Due to the limited availability of outbreak data and the paucity of data available on actual germ warfare attacks, system validation is invariably performed through simulation. Two main simulation approaches, both relying on normal baseline patterns of annual recurrent incidence of cyclic diseases, are used to simulate the changes in data patterns resulting from potential covert or naturally occurring infectious disease outbreaks.

In the first approach, data representing historical normal patterns of healthcare utilization and other syndromic data are used to define a baseline upon which simulated data are superimposed to represent hypothetical outbreaks of a desired length, transmission rate, and magnitude. Invariably, due to data availability and timeliness, simulations are limited to the representation of total number of visits, chief complaints, and ICD-9 diagnostic codes collected at emergency departments [14,15,17]. For example, Reis and Mandl [15] developed a model to simulate abnormal emergency department visits of patients with respiratory illness by infusing additional visits, with varying size, shape, and duration, on top of historical data.

Such a simulation model is simple to implement and allows for parametric testing of the surveillance system and assessment of its sensitivity, specificity, and timeliness to changes in adjustable system parameters (e.g., detection thresholds). The approach has been limited to date, however, to modeling temporal variations of a single data type in a fixed, single physical location, such as the time-series analysis of acute respiratory infection of pediatric patients reported during visits to the emergency department of a given hospital. Hence, the approach has been strictly used to test univariate temporal detection algorithms. It would be difficult to broaden this simulation approach to allow for spatial and spatio-temporal data analysis and include a broader range of data types, such as the representation of different segments of the population presenting different syndromes in geographically dispersed locations, and the inclusion of over-the-counter medication and school/work absenteeism data. This is chiefly due to the lack of concurrent availability of these data types for the same exact population, the lack of linkage among the data types (e.g., most emergency departments do not record a patient work ZIP code), and, most importantly, the need for explicit mathematical models that can simulate the interdependent effects of an infectious outbreak on these data types.

The second approach allows for the modeling of such complex interdependent effects. By using numerical mathematical models, an initiating event, such as a specific disease outbreak, drives the models to represent the temporal and spatial evolution as well as the effects of the selected disease. BioWar, a scalable multi-agent network model of the impact of weaponized biological diseases in metropolitan areas, is one of a handful of such simulation models [18]. Sponsored by the Defense Advanced Research Projects Agency (DARPA), BioWar uses historical data from a small number of actual cities (e.g., Pittsburgh, San Diego, Norfolk, and Hampton) using census, school tracking, and other publicly available information as baseline upon which the simulation models are applied. The simulation models allow for the representation of the city demographic distribution and associated risk factors for the modelled disease, wind dispersion, social network interactions, and mechanisms of disease transmission and time course, and provide their simulated repercussions in the population's symptoms and signs, death rate, diagnostic tests, school and work absenteeism, and over-the-counter pharmacy sales. BioWar allows for the representation of various pathogens, such as anthrax and smallpox, as well as naturally occurring epidemics. The weaknesses of this approach are that it can only model a fixed number of cities, does not allow for inter-city modeling of disease translocation, and unanticipated outbreaks, such as SARS, cannot be readily modeled to test the robustness of the detection algorithms to unknown diseases. More importantly, there are no benchmark data to validate the underlying assumptions and accuracy of these simulation models, questioning their value as a source of data to validate the detection algorithms.

6.0 U.S. MILITARY AND NATO SETTINGS

In this section, we discuss the unique geographic, demographic, and infrastructure attributes of military and NATO settings as well as the distinct sick-call procedures of military personnel and health-care providers that may affect the development, deployment, and usage of electronic medical surveillance systems.

6.1 U.S. Military Settings

Military populations and healthcare settings present unique challenges to electronic medical surveillance. Although fixed military medical facilities in garrison may have an infrastructure similar to their civilian counterparts, temporary facilities in deployed settings often have less robust communication capabilities. In addition, young healthy adults constitute a high percentage of the military population in an operational theater, while for many infectious diseases geriatric and pediatric age groups represent a more vulnerable population. Hence, syndromic surveillance systems deployed outside military installations could themselves serve as early

indicators for syndromic systems targeted to monitor military installations. Also due to demographics, an epidemic outbreak first occurring in deployed active-duty personnel could present in a less fulminant fashion than would be expected in the general population. Another relevant difference between military and civilian healthcare is the requirement for enlisted personnel to report to sick call if planning to miss work due to illness. Non-military personnel may miss work or school without having to visit a healthcare facility. Absenteeism may, therefore, be a variable marker during the time course of an epidemic outbreak, depending upon the population in which it occurs.

In some areas of the country, a prominent military healthcare beneficiary population coexists with the civilian population. In such regions, surveillance systems designed to serve these subpopulations separately would ideally work in concert. Integration of systems like the National Retail Data Monitor [19], which monitors civilian over-the-counter pharmacy sales, with an analogous method of tracking medication prescribing patterns from military healthcare facilities, would likely provide an earlier marker of aberrant activity in that region than either data stream alone.

Similar to what is occurring within the civilian healthcare community, a number of separate medical surveillance initiatives are underway within the U.S. military healthcare system [1,2]. To promote the development of complementary efforts and to ensure that such projects are directed at individual Service and DoD-level requirements, the Assistant Secretary of Defense for Health Affairs issued a policy memorandum in November, 2003 [20]. The memorandum specifies that all information management and information technology activities related to medical surveillance within the military are to be coordinated with the Deputy Assistant Secretary of Defense for Force Health Protection and Readiness and with the Force Health Protection Council. Also published in 2003 was the DoD Health Surveillance Master Plan, a report generated by the Integrated Process Team on DoD Comprehensive Health Surveillance. This plan details a phased approach to the development of an integrated comprehensive health surveillance capability for all of DoD. Proposed initiatives include establishing a Surveillance Fusion Cell overseen by a Board of Governors, with expansion to a Surveillance Operations Center and the eventual creation of an independent Surveillance Field Operating Agency with connections to the Department of Health and Human Services, Department of Homeland Security and other U.S. government agencies.

6.2 NATO Settings

To the naïve observer, the establishment of integrated medical surveillance within NATO would appear to be relatively simple—develop a system, field it, and issue orders to use it. In reality, the issue is much more complex. NATO does not have a single medical service for its deployed troops, and certainly does not have a consolidated one for its civilian population. NATO, as an organization, does not control medical services to its personnel and populations, although (combined with Partnership for Peace) it actually has 44 disparate national military medical services. Until 1997, all operational logistics (including medical care) in NATO were by doctrine a strictly national responsibility, without any NATO-level control. In that year, new doctrine was issued (MC 319/1) specifying that logistics support was henceforth a shared responsibility between the troop-contributing nations and the NATO operational commander. This new doctrine was then extended specifically to the medical environment (MC 326/1). In accordance with current doctrine, a NATO commander can require reports on, and inspect medical assets intended to support his/her operations. As required by NATO medical doctrine [21,22], medical surveillance can be seen as a shared responsibility between the nations and the Force Commander, but the details of how to accomplish that most effectively are still being developed. Medical units remain generally under national control, and hence, use national systems of medical records, documentation of care, and medical surveillance. The NATO mission in this context is to promote interoperability without compromising national sovereignty. The standard NATO mechanism for

accomplishing this requirement for interoperability is the NATO Standardisation Program, described in detail in Allied Administrative Publication 3 [23].

In 1996, EPI-NATO [24], the first attempt at a NATO multinational DNBI medical surveillance tool for use in deployed and garrison-based forces, was developed and fielded. A modification of the British J-95 system [25], it has been used intermittently in Bosnia and Kosovo. EPI-NATO collects and tracks initial and subsequent sick calls to a medical treatment facility, identifying patients seen by symptom complex, such as climatic injury, sports injury, respiratory disease, and dermatologic problems, rather than by diagnosis. Consolidated reports of the incidence of these symptom complexes are transmitted up the chain of command to allow early visibility of outbreaks, without waiting for definitive diagnoses to be made. When appropriately used and analysed, the system functioned as designed, and allowed the early identification of at least one significant outbreak of disease in Bosnia [26]. However, in the absence of an electronic medical reporting system and a reasoning engine or algorithm to detect abnormal events, EPI-NATO suffered from delays in detection, reporting, and transmission, since each entry and detection was made manually, and forwarding of reports required specific action rather than occurring automatically. Further, the personnel with epidemiological expertise necessary to use the data optimally were not made available in adequate numbers by the nations, and data analysis was never timely or complete. Thus, EPI-NATO failed to live up to its potential and some nations simply refused to use the system. Others felt it was duplicative of their national reporting systems. Although the use of EPI-NATO was directed in policy [21], implementation problems included a lack of written implementation policy and lack of integration with existing documents on medical reporting.

With the ongoing change in NATO's missions and its realization that both military forces and civilian populations are likely targets for attack, NATO has begun to actively pursue the development of an integrated medical surveillance system. The development of an on-line military and civil disease surveillance system is one of five NBC defense initiatives approved during a recent NATO summit meeting held in Prague. Many initiatives to address this identified need are now underway. Specifications and requirements for the development and deployment of surveillance systems, capable of reporting either symptom complexes or diagnostic information to a central repository, are under development. Also, efforts by the Joint Medical Committee to evaluate various national surveillance systems to identify mechanisms for integrating them into a fully-functional NATO surveillance system suitable for both military and civilian populations are slowly moving forward.

7.0 DISCUSSION

Major obstacles to syndromic surveillance data collection are the disparate information systems and data communications networks used by the DoD and its civilian health care counterparts. Medical information infrastructures are very often incompatible in hardware, software, data architecture, and /or data transmission protocols, even among the various government agencies and the individual military services. Even if existing and planned digital information systems for both the military and civilian communities could be integrated, there remain significant bandwidth challenges especially in the forward deployed military settings. Only recently, through implementation of various components of the TMIP, has the DoD medical information infrastructure been extended to forward deployed military forces, although significant bandwidth and information processing and infrastructure issues remain to be solved. A world-wide open system with network-centric medical information reference architecture, implemented among diverse government and civilian agencies via an approach like the DoD Global Information Grid [3], is essential to developing an effective "all-source" medical syndromic surveillance system.

Several challenges related to the selection and utilization of data sources have been identified. As data sources are chosen further along the timeline of epidemic outbreak detection, increased specificity is gained at the expense of decreased response lead time in a given community. Dissemination of specific diagnostic information from one community, however, could prompt other unaffected communities to assess their risk of acquiring the pathogen, potentially providing ample opportunity for response planning. Data management issues such as accuracy, accessibility, confidentiality, and lack of standardization also need to be addressed. Demonstration of the added value of electronic medical surveillance systems to public health will be key to proving cost-effectiveness, in particular, due to the significant resources required to procure and maintain the needed data acquisition infrastruture. In addition, defining the scope of response appropriate for a given type of alert, cognizant of the inherent resource limitations and minimal mobilization times associated with the response community, poses a significant challenge to their implementation and sustainability.

Challenges associated with near real-time outbreak detection methods abound. First, there is the common trade-off issue concerning sensitivity versus specificity of detection, which is reflected by the "appropriate" selection of alert threshold levels. Setting the thresholds too low improves timelines of detection and sensitivity (i.e., miss detection) at the expense of specificity (i.e., false detection), while setting the thresholds too high improves specificity at the expense of timeliness and sensitivity. As the optimal selection of threshold levels depends on the magnitude and time evolution of the outbreak, which are not known a priori, it cannot be realized. Second, the use of systems that employ geographic locations in their reasoning process are seriously impaired by the lack of the necessary data and ambiguity, as exposure location is not necessarily linked to where an individual lives, works, purchases medication, and seeks medical care. Next, metrics need to be generated and used to systematically and quantitatively measure the effectiveness of electronic medical surveillance systems against more traditional methods of detecting and tracking infectious disease outbreaks. Finally, due to the large amount of noise in syndromic data, the commonly accepted approach to detect outbreaks by comparing current observations with expected values may be conceptually flawed. During the initial stages of the 2003 SARS epidemic in Hong Kong, there was no detectable difference between the number of patients being registered, first believed to have contracted viral pneumonia, and the expected number of pneumonia cases for that time of the year [27]. The first useful hint that something unusual was happening occurred when a large number of health care providers themselves started to get sick. As a consequence, Hong Kong hospitals are now monitoring absenteeism among health care providers. While such an approach would be useful is detecting a re-emergence of SARS, it is unlikely that it will be valuable in detecting the "next SARS," that is, the emergence of a new, unanticipated disease for which transmission mechanisms are not known in advance.

Another key challenge is the quantitative validation of these systems. Simulation techniques currently available—to determine which outbreaks can be detected, how large they must be to be detected, and how early they can be detected—are inadequate, lacking the capability to model complex scenarios. They are either limited to model temporal variations of a single variable, e.g., the number of total daily emergency department visits, or are restricted to model fixed geographical regions and pre-specified outbreaks, such as anthrax and smallpox terrorist attacks in a given city. For instance, it is not possible to fully model the sequence of events of the anthrax attacks in the U.S. or those leading to the international SARS outbreak, preventing the validation of syndromic surveillance systems with the complexity of real-world events. Moreover, the lack of benchmark data precludes the validation of the simulation models themselves.

Differences between military and civilian populations and healthcare facilities should be kept in mind as electronic medical surveillance systems are developed and deployed. Disease presentations and the timeline for data sources used as markers of disease may vary between these groups, suggesting that a system designed for one may not be applicable to the other. From a U.S. military perspective, DoD has recently published a

policy memorandum and document intended to establish a management strategy for integrating the multiple currently separate initiatives directed at health surveillance within the services. From a NATO perspective, other than EPI-NATO [24], no NATO-wide surveillance system is currently functional or deployed. NATO as an organization has formally recognized the need to develop a medical surveillance tool that will provide early warning of disease outbreaks among deployed forces and civilian population, and is progressing in that direction. Since medical data in NATO has not previously been considered anything other than national data, security of medical data has strictly been a national responsibility. A new requirement has been identified to ensure that multinational procedures are set up to meet the privacy requirements of the most stringent nation participating, and to identify required data fields and reporting mechanisms. Issues, such as standardization of current systems, differing national policies on medical data protection, and the need for integrated multinational monitoring of the civilian population, have slowed the development of such systems.

8.0 CONCLUSIONS

There is considerable activity in many agencies of the U.S. government, academia, private sector, and, more recently, in NATO to develop electronic medical surveillance systems intended to provide early warning of bioterrorist attacks and naturally occurring epidemics from monitoring a wide variety of pre-clinical and pre-diagnostic data sources. Currently in various stages of development and evaluation, these independently developed systems display striking similarities in the types of data they collect and use, the underlying detection algorithms, and in their overall system architecture. They also share enormous technical challenges, such as the timely access of quality data from disparate sources, algorithms that lack the necessary sensitivity and specificity, and inadequate means for algorithm validation.

If ongoing electronic surveillance efforts within the U.S. and its NATO partners are to succeed, a refocused basic and applied research program is needed to: 1) identify and validate more reliable sources of evidence, 2) improve methods for data collection, standardization, and dissemination through an integrated open systems global infrastructure, 3) develop more effective analytic approaches and prediction algorithms, 4) broaden outbreak simulation efforts to account for introduction of unknown agents over wider regional areas of observation, and 5) identify more reliable validation methods.

In summary, the challenges of establishing an information processing infrastructure for timely collection of medical syndromic data seem to be essentially one of implementation. Conversely, the challenges in detection algorithms are more fundamental, requiring substantial research. Ideally, what is needed is a comprehensive computer system that can take in myriad types of data from multiple dispersed data sources and—through a combination of inductive, deductive, and non-monotonic reasoning algorithms that combine infection disease domain knowledge and evidential data—synthesize the data into useful information, such as a priority list of possible initiating events to be considered by military and public health providers. Research in outbreak simulation models also needs to be expanded. Agencies funding such efforts should require developers to make them open source. In the absence of real-world data for model validation, unrestricted availability would accelerate model development and allow the scientific community at large to review the underlying model assumptions.

Apart from these vast technical challenges, the practical relevance of medical surveillance systems for early detection of outbreaks in deployed and garrison-based military personnel and the general population is still to be demonstrated. It is unclear if such systems will be more effective than the traditional reliance on doctors, nurses, and hospitals to alert public health officials of a disease outbreak before hospitals are flooded with very sick patients. It is questionable if they can be tuned to be sensitive enough to identify abnormal patterns

and set off alerts when just a few individuals are infected, while limiting the number of false positive alerts. Assuming that the system detects an abnormal event, e.g., in the number of total daily emergency department visits, what information does it offer that military and public health providers can act on? As recent outbreaks have shown, knowing only that an abnormal event is occurring without having more specific information, such as an understanding of the mechanisms of disease transmission, is not likely to lead to timely and effective actions that can reduce morbidity and mortality rates. In the short term, we need to better understand the capabilities of these systems. One possibility, is to generate and use metrics to systematically and quantitatively measure the effectiveness of deployed systems against more traditional methods of detecting and tracking infectious disease outbreaks.

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DISCLAIMER

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SYMPOSIA DISCUSSION - PAPER 12

Authors Name: Dr Reifman (US)

Discussor's Name: Major Gillen (US)

Question:
The model on page 5 does not reflect the entire scope of health surveillance. Specifically, the integration of occupational and environmental surveillance should be infused to the first two areas of the diagram. Therefore broadening the scope to include, monitoring before the impact is being experienced by human exposure – illness onset.

Author's Reply:
Agree. Risk assessment data are already being used/proposed in syndromic surveillance systems.

Medical Surveillance for a Soldier Centered Battlespace Awareness

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ABSTRACT

Recent advances in sensor technologies have enabled a net centric view of the battlespace, substantially increasing situational awareness for the warfighter. However, this net centric awareness has yet to be extended to the status of the warfighters themselves. Medical technologies have progressed to the degree that portable, rugged and wireless designs can be conceived of that could give coalition commanders and medical personnel a view of the health and well being of their troops. This could give coalition forces a distinct advantage in both urban ops and more traditional force protection situations, allowing them to place the right resources in the best location well ahead of the usual indicators. These kinds of technologies would enable coalition commanders to make informed choices about when and where to send their troops, and how many may be prepared for the next mission. In the era of "just in time" force delivery – this capability will enable the best use of coalition forces in the events and actions that they are likely to face in the future. This paper will discuss the latest technologies under development that can be applied to the medical surveillance problem. In addition to technologies for the individual soldier, information delivery and display at the level of the commander and senior medical personnel will be discussed. As sensor presence on the battlefield increases, so does the need to manage and control the flow of information to enable the most effective decision making. It is only through the integration of the field based picture with the conditions and status of the warfighter that commanders will have a truly integrated vision of the battlespace.

1.0 INTRODUCTION

The NetCentric Battlespace is a pervasive and defining force in the current concept of Coalition operations. Technologists are increasingly focused on new sensors for the battlefield and innovative means of deploying them for the richest depiction of the battlespace in real-time. However, there is one aspect of the sensor

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enabled battlespace that has been largely ignored – the soldier centered battlespace. What is meant by "soldier-centered"? It is without question that our human assets are some of the most valuable that we have in the field. In the ever present reality of reduced manning, our warfighters have been asked to carry out more and more tasks – without much concern for the impact on their performance or moment to moment status. This is not to say that soldiers have lacked enhancements in their daily operations. Certainly protective gear has improved, soldiers have been provided with more sophisticated equipment to navigate in day and night, and warfighters have access to advanced communications tools from their remote field locations. However, the concept of the soldier centered battlespace centers around the notion that the soldier himself can be physically instrumented with sensors in order to give an accurate picture of their real-time status and readiness. These sensors would not provide information about the battlespace picture per se – however they would provide valuable information about the status of the human assets on the ground, therefore including them as a node in the networked operational picture.

2.0 STATUS MONITORING OF THE INDIVIDUAL SOLDIER

2.1 Medical Monitoring

2.1.1 Soldier-Based Assessment

Within the deployed environment there are several levels of surveillance that could be proposed. Some with a strictly medical assessment focus are already under consideration in large service sponsored efforts. These involve equipping the individual soldier with shirts or other clothing items that can communicate real-time information based on the integrity of the shirt and recordings from its embedded sensors. (see: http://www.gtwm.gatech.edu/ for details). These shirts can be equipped with a variety of sensors, including ECG (electrocardiogram), temperature, respiration rate, and a penetration monitor to detect wounds from ballistic objects on the torso of the soldier. These data are all collected and relayed to a status monitor that is worn by the individual soldier – which wirelessly communicates the real-time information to a remote medical unit. This is a significant advance for casualty care and the immediate deployment of these technologies to the battlefield would be of enormous value to both soldiers and those that manage casualties in the field. These sensor combinations would allow for rapid alerting and assessment of a soldier's wounds, and would accelerate their care and treatment – either in the field or at an advanced casualty care center.

The availability of soldier-worn sensors has also led to designs that utilize ultra low noise electronics for sensing and amplification of biological signals. One effort currently underway is developing a non-contact electrode that can work through everyday clothing – no special fabrics or fibers. These researchers have proposed that the electrical signals emanating from the skin are the major source of noise contamination in biological signals. Thus, they developed a novel non-contact sensor that detects the electric field emanating from the body, completely eliminating the contaminating signal from the skin. The first designs have detected ECG signals, but in addition to heart rate measures, they are now working on an EEG (electroencephalography) design that could be used to detect a variety of medically relevant signals without touching the operator at all. The sensor has been miniaturized, down to the size of a dime and able to be casually integrated into regular clothing items like t-shirts and helmets. The sensors are inexpensive and entirely passive, and work is underway to make the electrodes more sensitive to biological signals, wearable and wireless.

2.1.1 Novel Portable Assessment Tools

In addition to soldier-worn technologies, efforts are now underway to use the latest in optically based technologies for medical assessment in the field. Considerable effort has been made to protect our warfighters from head injury. However, closed head injuries are still a major concern in the dangerous work environments soldiers face when stationed at sea or in the battlefield. Even in non-military urban settings, acute head trauma is a leading cause of death and injury. The emergence of subdural/intracranial hematomas following blunt force head trauma can lead to life threatening complications including brain damage, disability and death particularly if the injury is not detected immediately after an event. An acute subdural hematoma (SDH) is a collection of rapidly clotting blood below the inner layer of the brain membrane (dura) but external to the brain. In operational or remote settings, immediate evaluation of these injuries following a closed head event is limited to the assessment tools at hand. Ships at sea and forward deployed hospitals are not equipped with technologies such as magnetic resonance imaging (MRI) and computer aided tomography (CAT) scans. These technologies are the standard tools for determining the presence of subdural hematomas – but their excessive size, lack of portability and cost prohibit their use in operational environments. Near infrared wavelengths of light can be used non-invasively to penetrate through the skull and detect/image blood beneath the skull surface and in the cortical layer of the brain. This affords combat casuality care medical teams an opportunity to "see" below the skull and make rapid assessments of patient status. Devices using near infrared spectroscopy have the advantage of being relatively inexpensive due to recent advances in near infrared sources and detectors. The development of imaging devices using near infrared spectroscopy will offer the military medical community an opportunity to detect subdural hematomas on site and should afford medical workers more time to make critical decisions about whether or not a patient needs to be evacuated from their current location.

2.2 Advanced Status Monitoring

In addition to purely medical monitoring, we now have the opportunity to begin incorporating more complex sensors into the baseline status monitoring systems described above. As discussed in the NATO RTO HFM-056/TG-008 Report on "Operator Functional State Assessment", there are a myriad of sensors now in development that can give an even more complete evaluation of real-time soldier status. In this context, soldier status does not just refer to the physical integrity of the warfighter, it refers to the cognitive or mental capacity of the warfighter as measured in the real-time operational environment. When we consider this broader definition of status – we can look at cognitive measures, such as workload, mental fatigue, stress, sleep deprivation and even begin to associate measures with concepts like situational awareness. Current investments in these technologies have contributed to revolution in this area and a significant paradigm shift with respect to the types of data that can be collected in real-time. Although not all of these tools are currently ready for operational deployment, the following table lists some of the sensors in use:

Actigraphy	Actigraphy
Blood Flow	
BP	Blood Pressure
Core Temp	Core Temperature
ECG (HR,HRV)	Electrocardiography
EDA	Electrodermal Activity
EEG	Electroencephalography
EMG	Electromyography
EOG/EyeMovement	Electrooculography
fMRI/imaging	Functional MRI
Hormonal	Hormonal
NIRS	Near-Infrared Spectroscopy
Oximetry	Oxygen Saturation
Respiration	Respiration Parameters

Table 1: Types of Physiological Monitoring Available

Of the measures listed in Table 1, not all are associated with a cognitive or mental state. However, all of these sensors have been evaluated to some degree for their usefulness in characterizing the warfighter's state. The author's of this paper recommend the NATO Report (RTO HFM-056/TG-008) for a complete evaluation and discussion of these sensors in primarily the aviation domain. Work is now underway to evaluate these measurements in the mobile environment and under increasingly hostile conditions. The eventual goal of this work is two fold. One aspect is the improvement of human performance through the assessment of soldier status in real-time. With this knowledge, information systems can be designed and modified in order to present information to the warfighter in the most beneficial and least stressful manner. In addition, a side goal of this work is the development of tools to be used in order to conduct 'fitness for duty' evaluations in real-time. This is part medical – but also part cognitive – as a warfighter may by physically ready for battle, but may not cognitively or mentally be in the zone. The hope is that preventative use of these technologies would reduce battlefield causalities, non-combat related mishaps, and other events that are due to mental fatigue. Circumstances in the field may necessitate soldiers to perform at sub-optimal levels; however, these technologies would give medical personnel the opportunity to exercise preventative care instead of triage.

3.0 MEDICAL SURVEILLANCE OF UNITS OF ACTION

The surveillance discussed in this paper thus far has centered around the individual solider and the assessment of his or her status in real-time. However, an even more powerful application of these tools could be used at the level of the medical officer or battalion commander to establish a real-time awareness of the health and status of their troops. This begins to approach the soldier centered battlespace awareness concept initially discussed. One could envision a whole range of status reporting – from a basic "green, yellow, red" readiness system that would be emitted by each soldier's personal status monitor giving a real-time picture of troop status - to even more complex and diagnostic data – particularly for medical officers – about the fatigue, stress, hydration status and physiological health of the troops. This type of information could give coalition forces a distinct advantage in the operational setting, allowing them to place the right human resources in the engagement at the right time. These kinds of technologies would also enable coalition commanders to make informed choices about when and where to send their troops, and how many may be prepared for the next mission. This is a notional concept at this time, but this type of capability should be considered as soldier-based measurements become part of the operational picture.

4.0 INFORMATION MANAGEMENT IN THE BATTLESPACE

Finally, we would be remiss without mentioning the impact that this additional information will have on workload in the field. Arguably, each additional node in the netcentric battlespace requires someone to receive process and interpret that information. Adding humans to this picture, through their assessed status is no different, and will require an information management solution for commanders and medical personnel to handle the flow of information. There is probably a great deal that can be learned from the civilian medical community, and the new emphasis on telemedicine. However, this field is expanding rapidly, and the development of new technologies for monitoring is likely to outpace the development of easy to use interfaces to fuse and analyze data to convert it to actionable format. Thus, these authors recommend that resources be invested in the human-computer interfaces that will collect this information – so that this approach does not become clouded by information overload rendering the data unusable in the field environment

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SYMPOSIA DISCUSSION - PAPER 13

Authors Name: Dr Schmorrow (US)

Discussor's Name: Col Cox (US)

Question:
Sensor data includes most, if not all, of the physiologic parameters used in polygraphs. This could be of potential value when needing to identify individuals communicating under duress, e.g., individual is at enemy gun point, but his comrades are unaware. Has such an applicant been considered? If so, what validity might you expect/predict? The application was not designed for that purpose, but multiple applications are possible using this type of sensor data. There is a problem of distinguishing between lie, deceit, or "simple" physical stress.

Author's Reply:
This technology will be able to detail levels of stress as well as associated bio markers of confusion and disorientation.

Authors Name: Dr Schmorrow (US)

Discussor's Name: Prof. Dr Garland (US)

Question:
1) What is the time-line for Sensors and algoriths for Medical Situation Awareness in theater?

   For:

   a. Personal status – normal situation

   b. Injured individual – assessment/monitoring

2) Is it reasonable to add these technologies in a 5 year time frame?

Author's reply:
1a) Personal status monitoring capability in fixed facilities will arrive in 3 to 5 years, in the field will arrive 7 to 10 years.

1b) Injured individuals. This capability will be demonstration as a portable low cost technology in 24 months.

2) Availability to medical facilities in a broader fashion will be available in 3 to 5 years.

A New System of Automated Eco-genetic Database and Modern Conception of Prognosis of Bronchial Asthma

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ABSTRACT

The high epidemiological indexes of bronchial asthma (BA) and the high frequency of BA formation in early age groups, multiple genetic and external factors of phenotypic expression dictate the necessity of integrative study of eco-genetic characteristics of BA.

At present, it is widely recognized that hereditary predisposition is the main factor for the formation of BA. A unified assessment system of the cumulative impact of ecological and endogenous factors of BA does not exist on level of population or the individual.

The essential problem of modern medical prognosis is to work out an informational prognostic system.

We made formalized cards of BA primary and secondary prognosis based on the revealed complex of ecological, onto-genetical, clinical-laboratory and genotype and phenotype predictors.

The automatic prognostic system is formed by use of algorithm (consecutive statistic analysis of similar sings) according to the diminishing order of summary characteristic of predictors informational indices.

New and significant characteristic of the work is that based on the structure of ecological risk (nature of operation, genesis, intensity - attributive risk, duration of impact - cumulating risk) and genetic markers

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(genealogical data, AB0, HLA and PI-genetic systems and other phenotypic markers) of BA, the formalized cards are recommended for the purpose of BA secondary prognosis differentiate for girls and boys.

The resulting automated assessment new system will allow carrying out processing personal data of genotypic and phenotypic characteristics and creating achievable, economical diagnostic and prevention methods of BA based on formalized cards.

The analysis of the data obtained has shown the relationship between the increasing prevalence rate of allergic diseases in children and the chemical air pollution intensity ($p<0,001$). In the districts with high air pollution intensity the earlier onset, heavier development of allergic diseases in children and the increase of the polyvalent sensitization frequency ($p<0,05$) have been recorded.

Thus, the obtained results indicate a significant influence of the air composition on the prevalence of allergic diseases and its growth with the pollution intensity in the dwelling district.

Problem Statement

Bronchial asthma problem has very high medical and social importance. At the same time still is very difficult to prevent this disease despite of good knowledge of Bronchial Asthma's(BA) pathogenesis and triggers of attacks. Sometimes doctors are unable to manage well this pathology, because of neglected early diagnostic. According to the most actual and perspective direction of nowadays allergology it is working out new scientific approach of resolution of bronchial asthma problems in young population.

The high epidemiological indexes of BA and high frequency of BA formation in young age groups are occurred in the last period in Georgia as well in the whole world. Influence of multiple factors on prevalence of disease: influence of external and internal risk-factors, genetic predisposition to developing BA dictate the necessity of integrative study of ecological-genetic study of BA.

At present it is widely recognized that hereditary predisposition plays the most important role in the developing of disease. But hereditary predisposition is not everything recognizable by physicians because it is not demonstrated by clinically manifested asthma in parents and other close relatives of affected individual.

Hence to above mentioned, reveal of young individuals having maximum risk of BA is the milestone of preventive measurements and early diagnostic of BA. It means that we talk about prognostic of BA among young individuals who have not any symptoms of disease before and according their medical history they are practically healthy. Primary prognostic of disease like BA is early diagnostic in fact. This is priority approach of Public health.

Georgian Armed Forces is been forming now. One of the most important problem of nowadays military medicine very high morbidity of military personnel. Despite the fact that the scale and quality of conscripts' medical investigation, that is being done at the moment of recruiting, is far from required standards, - above mentioned morbidity level is a little bit unexpected.

We have done randomized investigation of patients' histories in Central Military Hospital (Tbilisi) and Military Outpatient Clinic (Tbilisi) for the last 5 years period: 1998-2003. Among the frequently revealed diseases, are allergic disorders: urticaria, Quinke's oedema, chronicle rhinitis, conjunctivitis and bronchial asthma. Above mentioned diseases often became the causes of soldiers' retention from the army. Their frequency are as well high in the civil population of similar age group.

In conditions of a substantial increase in bronchial asthma incidence rate epidemiological and investigations become very important. It should be mentioned that none of the official documents of WHO and European international societies gives information on epidemiological investigations carried out in the former USSR. This can be explained, in official data such as low level of the bronchial asthma incidence rate is given that this figures are regarded as rather doubtful. In order to obtain impartial information on bronchial asthma in adolescent and young population in Tbilisi a prospective epidemiological investigation has been carried out.

Materials and methods

It was investigated 184 families, among them: 171 young persons, aged 12-20 years with Bronchial Asthma, 26 sybses and 295 parents. 134 young patients were investigated with deep medical (specific allergologic) investigation:

1. Allergologic anamnesis was studying by special scheme elaborated in Russian Scientific Research Institute of Allergology
2. Allergic skin tests: was performed according above-mentioned Institutes' recommendations
3. Study of individuals' immunologic status: determination of T-Lymphocytes subpopulations by monoclonal antibodies by using laser fluoro-cytometric method, determination of IgA, IgM, IgG, was performed by using method of radial immunes-diffusion, and total IgE – by using radio-immunological method
4. Program providing : CH program language, using R:BASE interface
5. Clinical-genealogic method (Falconer D., 1965): study of genealogical tree of investigated families.
6. Choosing of markers: it was used to approaches: 1. seeking of phenotype markers, that is characterized with high significance of mathematical magnitude and 2. seeking of genotype markers that is genetically associated with disease.

I. Investigation of genotypic and phenotypic markers:

1. Erythrocyte markers: ABO, Rhesus systems was determined by standard serums,
2. Leukocyte markers: HLA system's markers were determined Terosoki's (micro –lymphocyte-toxic tests) typing was performed with 1st class (A, B, C) antigens and 2nd class (DR, DQ) locus's
3. Phenotyping of PI genes was performed using iso-electric focusing in poly-acrilamid gel
4. Anthropometric investigations:
   - **a.** Height/weight ratio at birth
   - **b.** Eye's and hair's colour: it was determined three basic types of eye colour: bright: blue, grey; Transitional: green, greenish-brown; Dark: black, dark brown. and three basic hair colors: bright: blond, light chestnut, dark chestnut, black;
5. Phenotype signs:
   - **a. Morphologic signs**:
     - i. Pinna's lower lobe type,
     - ii. Type and location of vortex on the head: it exists six types of this sign: 1. centred and clockwise directed, 2. centred and counter clockwise directed, 3. located at right and clockwise directed, 4. located at right and counter clockwise directed, 5. located at left and clockwise directed, 6. located at left and counter clockwise directed.
     - iii. Dermatogliphical data: picture of palm lines.
   - **b. Physiological signs:**
     - i. Ability to turn a tong into tube
     - ii. Type of finger's crossing: left and right type
     - iii. Hand's crossing type: left and right type
   - **c. Ear-wax type:** it was determining by otoscopic method:
     - i. Dry ear-wax type

ii. Wet ear-wax type

d. Stigmas: minor congenital, multi-factorial defects. They rarely are caused by teratogenic action of drugs.

II. Method of genetical ans statistical analyze;

III. Method of multi-dimensional phenotyping: algometric- hierarchic(al) cluster analyze of chosen systems of signs. Analyze was performed in the interactive order.

By this method we have determined four types of phenotype classes:

1. Classes of basic predisposition, including up to 80% of individuals with manifested asthma;
2. Classes of relative predisposition, including less then 80% of individuals with manifested asthma;
3. Phenotype classes including a few patients
4. Phenotype classes not predisposed to BA, basically including healthy persons.

Results and discussion:

Questioning and medical examination have shown that in the investigated contingent 184 persons have clinically manifested BA with different severity. Among them - 119 (65,4%) were males and 55 (35,6%) - females. 32,7% of patients had severe BA, 62,6% - moderate BA and 4,7% - mild BA. Investigation of this groups revealed that all significant risk factors of BA were present: atopic diathesis, nutritional allergy, drug-induced allergy, asphyxia of foetus or newborn child, pathological pregnancy and pathological delivery.

Fig 1.

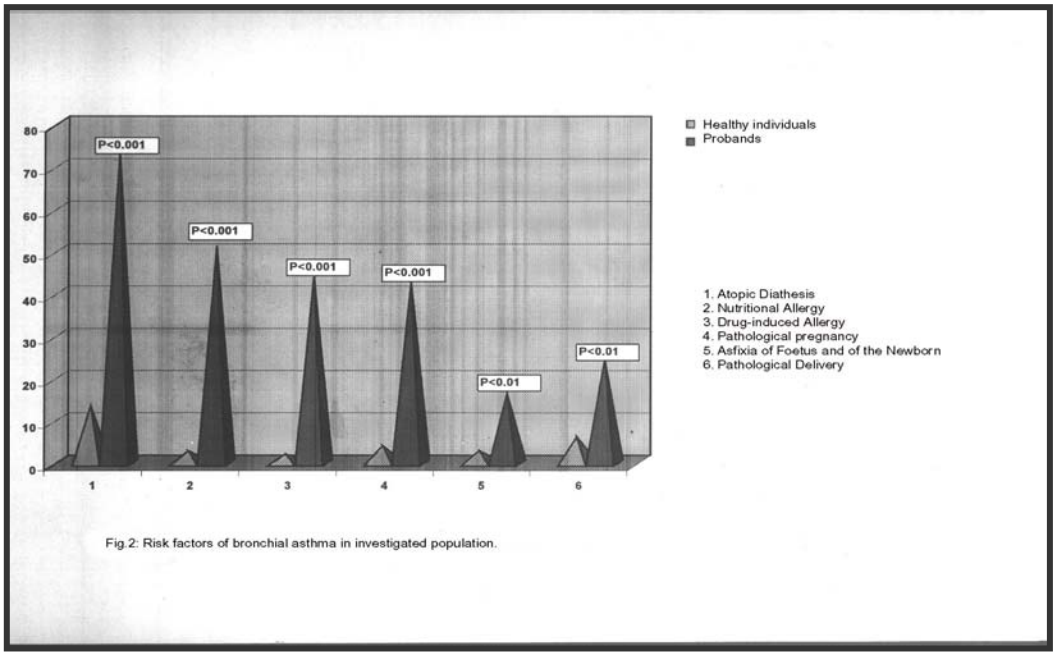

Fig.2: Risk factors of bronchial asthma in investigated population.

Phenotypic characteristics of investigated contingent showed that young persons with BA have the following phenotypical peculiarities: 1) High height-weight indexes at the birth moment: M^+ (13,7% in the basic and 7% in the control group, P<0.001), and M°/ M^+ (39,5% and 17,1%, P<0.01); 2) Fair hairs (29,4% and 20,7%, P,0,01): 3) Bright eyes (24.4% and 15,5%, P<0.001); 4) Absence of ability of turning

tongue into tube (66,3%, P<0,001), 5) Type of vortex on the head: centered and anticlockwise directed (44,1%, P<0,001) and located at right and anticlockwise directed, 6) Dermatoglifiks: significant rising of frequency of ulnar stitch encountering (63,5%, P<0,005), absence of "C" three-radius (11,0%, P<0.01) compare with control group. Besides dry earwax gene encountering in investigated group have significantly increased, whereas wet earwax gene encountering was twice more less in healthy persons. There was revealed significant positive BA predictors, that are associated with disease, particularly 0 (I) of ABO group (RR=1,75,P<0.01) It was revealed high authentic positive association of BA with antigen B7 (RR=15,12; P<0,001) and DR5 (RR=8,42, p<0,0001) .

Fig. 2

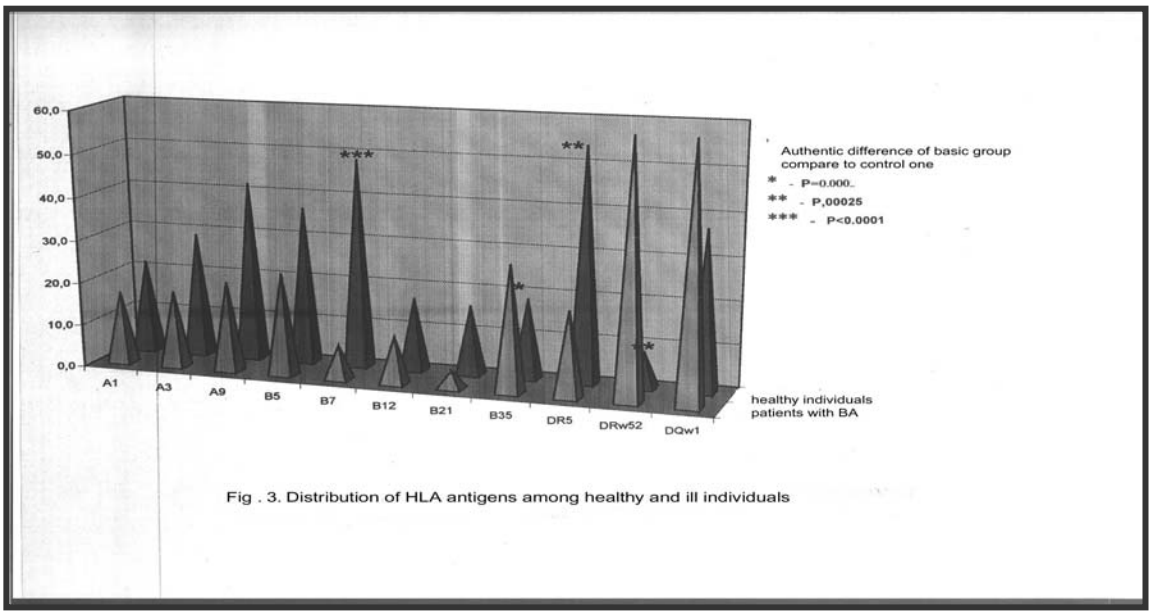

Fig . 3. Distribution of HLA antigens among healthy and ill individuals

Fig.3

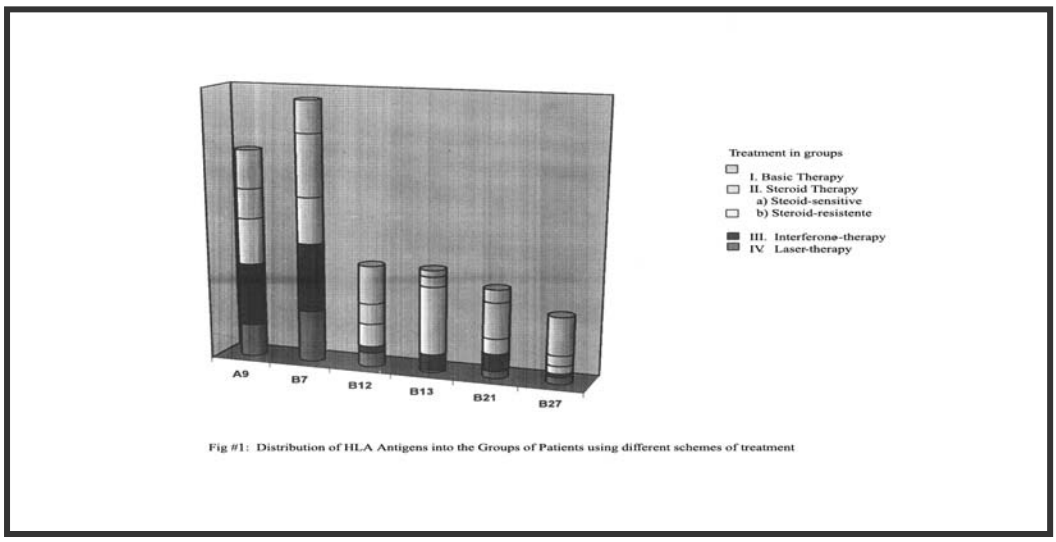

Fig #1: Distribution of HLA Antigens into the Groups of Patients using different schemes of treatment

In the table #1 is shown the tabulation of the significant phenotypic signs.

table #1 **Volume and Quality of Performed Investigation in the Targeted Contingent**

#	Performed investigation	Investigated Contingent			Total number of investigated persons
		Probands	Parents and Sybces	Control group	
1	**Clinical and Genealogic Investigation**	184	321	793	505
2	**Anthropometry:**	212	1002	793	2007
	a. height and weight indexes at the birth moment	212	1002	793	2007
	b. color of yes and hairs	184	321	116	621
	c. location of the vortex on the head	184	321	116	621
	Phenotypical Indexes				
3	**Morphological:**				
	a. Pinna's lower lobe type	184	321	116	621
	b. dermatoglifics	184	321	116	621
4	**Physiological:**				
	a. ability to tube a tongue	184	321	116	621
	b. type of hand crossing	184	321	116	621
5	**Stigmas**	184	321	116	621
	Genes markers				
6	**Type of earwax (cerumen)**	184	321	116	621
7	**Erythrocytes Antigen System:**				
	a. ABO system	220	1002	793	2015
	b. Rhesus system	220	1002	793	2015
	c. MNS system	96	321	214	631
	d. P system	98	321	214	
8	**Leukocyte Antigen System:**				
	HLA system	117	-	200	317
9	**PI – Phenotyping**	117	15	200	332

For establishment of phenotypic structure of population using multi-dimensional phenotyping it was determining for each predictor: statistical index between healthy and ill individuals (t-criteria by Student). genetic association of signs with BA, heredity factor (h^2) and integrity of markers.

Wile resuming investigation results, it have to be mentioned that the worked out computing table is enough simple and opportune (handly) for comprehensive use. It is proposed, for primary prognostic of BA, to practical doctors working in outpatient clinics and dispensaries, as well for military doctors working with enlistment committee or in military units. This method gives the possibility for prognosis predisposition to bronchial asthma in young population.

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SYMPOSIA DISCUSSION - PAPER 14

Authors Name: Prof. Karseladze (Dr Goderdzishvili speaker) (Georgia)

Discussor's Name: Unknown

Question:
Did you investigate use of cigarette smoking in participants and family members?

Author's Reply:
We did not investigate use of cigarette smoking, because the aim of work was to evaluate the role of genetic and phenotype markers and their combinations in early diagnosis and prognosis of BA (Bronchial Asthma). But we plan to continue to study the problem of COPD in military personnel. In the initial questionnaire there are questions included concerning cigarette smoking. Cigarette smoking is an external risk-factor and trigger of COPD.

Authors Name: Prof. Karseladze (Dr Goderdzishvili speaker) (Georgia)

Discussor's Name: Dr Barrett (UK)

Question:
Are any steps being taken either by Government or Industry to reduce level of pollution in highly polluted areas?

Author's Reply:
No – but with our new Government comes a promise to be more attentive and we are going to present our results to national authorities. We hope our recommendations will influence on their decisions to improve the ecological situation in Tbilisi.

Satellite Interconnection of Military Hospitals of the SEDM Countries (SIMIHO): A Novel Technological Forum as Model for Military Medical Surveillance and Response in SE Europe

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SUMMARY

Introduction: Ministers of Defense (MOD) of AL, BU, CR, FY, GR, IT, RO, SL, TU, US under the auspices of the South East Europe Defense Ministerial Process (SEDM) have adopted on Oct 9th,2000, the Greek proposal for Satellite Interconnection of the MIlitary HOspitals (SIMIHO) of their major Military Hospitals, and this initiative is evolving into a state-of-the-art medical surveillance system. Materials and Methods: The SIMIHO Working Group (SIMIHO WG) was formulated, tasked to provide feasibility recommendations for the next MOD meeting, constituted by senior military medical and technical experts of all SEDM countries and convened in Athens on three separate occasions,.as SIMIIHO/Med WG, specifying the essential requirements for the network. The MOD in their Antalya meeting (Dec 2002) commanded that the SIMIHO WG will proceed into buying the essential material for the implementation of the project, and the work continues under the SIMIHO/Tech WG. Results: Within a time frame of 18 months, SIMIHO WG examined discussed and defined the following: (a) Compatibility issues of existing information infrastructure, including integration issues, (b) Telemedicine centers to be included in the project throughout SEDM countries. (c) Medical specifications

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for the SIMIHO Telemedicine integrated system, which may include telediagnosis, teleconsultation (videoconferencing) and medical teletraining. (d) Operational policy issues of the Telemedicine Network under construction. (e) Technical specifications, which among others include: utilization of a satellite network (avoidance of "single point failure"), a minimum of 2Mbps for interactive communication, and techniques for maximization of imaging performance with minimization of bandwidth utilization, and (f) Financial policy issues, such as possibility for "pay-per-use" bandwidth utilization. These proposal was adopted by the MOD Ministers and work is under way for the implementation of the Project, and possibly expanded into mobile rapid deployment force units, such as SEEBRIG in Sofia, BU. Conclusion: The above framework may serve as a model state-of the art medical surveillance system, which owns the potential to be utilized in several ways (e.g. C3, medical planning, casualty management) both in mobile units, and in case of civilian events, such as a major catastrophe with mass destruction.

1.0 INTRODUCTION

1.1 South East Defence Ministerial (SEDM) Process

South East Defence Ministerial (SEDM) Process constitutes a diplomatic initiative of the Ministers of Defence of the Countries (both NATO and PfP) occupying the sensitive area of the South East European territory (mainly Balkans and surrounding territory – ***Figure 1***), which aims to establish co-operation, understanding and communication among nations and countries, and therefore promote peace stability and better relationships through common projects, work and military action. The Countries participating in SEDM are (in alphabetical order): Albania (AL), Bulgaria (BU), Croatia (CR), the Former Yugoslavic Republic of Macedonia (FY), Italy (IT), Germany (GE), Greece (GR), Romania (RO), Slovenia (SL), Turkey (TU), and the United States of America (USA).

Figure 1: The South East Defence Ministerial (SEDM) Process Countries

The SEDM MOD meeting goals are facilitated by the SEDM Co-ordination Committee (SEDM-CC), whereas each if the SEDM Countries participates via a permanent delegation, and convenes at 3-monthly intervals.

1.2 The Satellite Interconnection of the Military Hospitals of the SEDM Countries (SIMIHO) Project and SIMIHO Medical Working Group (SIMIHO/Med WG)

During the first Ministers of Defence (MOD) Meeting held in Thessaloniki, GR, on Oct 9th, 2000, the Ministers adopted a proposal by the Greek MOD recorded in the Meeting minutes (Ref 1) as follows:

"*The ministers also endorsed the Greek proposal for The ministers also endorsed the Greek proposal for satellite interconnection of military hospitals of the SEDM Countries in order to practice telemedicine and exchange medical information. For this purpose, they decided to establish an experts group to examine the matter and to prepare recommendations for review by the Ministers of Defense.*"

Following the order above, the Satellite Interconnection of the Military Hospitals of the SEDM Countries (SIMIHO) Project was initiated, and the SIMIHO Medical Working Group (SIMIHO/Med WG) established, consisted of senior Medical Experts in Telemedicine as well as Computer and Telecommunications military experts and civilian counsellors, under a Greek Chairman.

1.3 NATO is nowadays involved into a operational continuum which spans from garrison operations to peace keeping and enforcing, up to war itself. Such a state-of-the-art network may well serve as a technological model for such a flexible medical surveillance system, which combines *per se* independency of terrestrial network, therefore deployability, cost effectiveness, simplicity and flexibility according its agreed operational policy.

This paper **aims** to present and discuss the experience of the SIMIHO Med WG in its efforts to set up principles, policy of a satellite facilitated network and address diversity among the ten (10) SEDM countries.

2.0 MATERIALS AND METHODS

2.1 SIMIHO Med WG Program of Work

This WG convened on three separate occasions within a 18-month period (2000 -2001): Dec 13th, 2000 – Mar 26th, 2001 – Sept 4th , 2001, resulting in outlining the appropriate technical and operational specifications of a satellite facilitated medical network, and a final recommendation report was promulgated to the 2nd SEDM MOD Meeting held in Antalya, TU, on Dec 20th, 2001.

The SEDM MOD Meeting adopted the recommendation as follows: "*The Ministers noted receipt o fthe progress report on Satellite Interconnection among Military Hospitals (SIMIHO) Working Group and supported continuation of the project*" (Ref 2).

2.2 SIMIHO/Tech WG Program of Work

Having defined the technical and operational specification of the medical network, the necessity arose for a new WG, tasked to acquire, install, and maintain the system defined: Therefore, SIMIHO Med WG evolved into SIMIHO Tech WG, whose Terms of Reference were approved by SEDM-CC on October 28th 2002, under silence procedure. This WG met in Rome, IT on November 27th 2002, in Athens, GR, March 20th 2003, in Rome, IT on June 27th 2003, and Sofia, BU on Sep 30th to Oct 1st, 03. The outcome of this WG is the preparation of an MOU to define operational policy and possible hardware/software alternatives.

3.0 RESULTS

As results of the SIMIHO Tech WG area still under negotiation, this paper will report on the results obtained by SIMIHO Med WG, in order to clarify specifications of the network system model.

Table 3: Technical Specification for Communication Infrastructure

1. One (1) Satellite terminal
2. Special modems for interconnection to computer infrastructure

3.1 SEDM Parties were able to designate a suitable military hospital for the SIMIHO project purposes, as shown in Table 1.

3.2. In all hospitals designated for the SIMIHO project, there was complete software compatibility among apparatuses possible to utilize in the project.

3.3.

	COUNTRY	ESTABLISHMENT	LOCATION	REMARKS
1	Albania	Central Military Hospital	Laprake, Tirana, AL	
2	Bulgaria	Military Medical Academy	Sofia, BU	
3	Croatia			No military Hospital
4	FYROM	Military Hospital	Ilidenska b.b. 1000, Skopje, FYROM	
5	Greece	251 Hellenic Air Force General Hospital.	Athens, 115 25 GR.	
6	Italy	Policlinico Mlilitare Di Roma.	Rome, IT.	
7	Romania	Clinical Emergency Military Hospital	Bucharesti, RO.	
8	Slovenia			No designation
9	Turkey	General Staff Health Department	Ankara, TU.	
10	USA	National Naval Medical Centre	Bethesda, Maryland, USA	

Table 1: Military Hospital designated in SEDM Countries for the SIMIHO Project

Table 2: Technical Specification for the Telemedicine Unit Equipment

---

1. One (1) Workstation with telemedicine software

1.1. Minimum Hardware Requirements

1.1.1. Processor Pentium III 1 GHz or Double processor Pentium III 700 MHz
1.1.2 Motherboard (supporting the selected processor)
1.1.3. Network board 10/100 Mb
1.1.4. SVGA card 64MB
1.1.5 Video capture card (recommended Osprey 100)
1.1.6. Sound Card
1.1.7 128 MB RAM
1.1.8 18 GB Hard Disk
1.1.9. CD/DVD-Rom
1.1.10. Headset – Microphone & Loudspeakers
1.1.11. Live Camera(-s) equipped with remote control (e.g. Canon CV-3)
1.1.12. Document camera(-s) (e.g. Canon CV-3)
1.1.13. Windows NT Workstation Software

1.2 Functional Characteristics of telemedicine software

1.2.1 Customised advanced telemedicine software: Medical applications (e.g. teleconsultation) require a high-end, interactive video conference system providing high quality real-time video, still-images and audio transmission.

1.2.2. *Multiple Video input* **:**

1.2.2.1. Live-cameras for true live video conferencing. These are cameras connected to different, medical equipment: room camera for open surgery (integrated into the operating light), surgical microscope camera, laparoscope and endoscope camera, camera of the pathological microscope etc.

1.2.2.2. Document-cameras: Scanned images from a live camera that are sent separately, e.g. CT, MRT, US images.

1.2.2.3. Virtual-camera: Sending of image-files (stored in TIFF, BMP, JPEG, GIF, etc format) previously saved to the computer or a network file server.

1.2.2.4. Adjustable video-window size: The user can alter the size of the Self-View window sent to the video-conference-partner, e.g. 128x96 pixels, 176x144 pixels etc.

1.2.2.5. Adjustable bit rate & frame rate - Use of scalable bandwidth transmission channels (starting from 128 kbps at least)

1.2.2.6. Remote pointers.

1.2.2.7. Optimized video compression codecs based on the concepts of Partition, Aggregation and Conditional Coding or future developments.

2. Off-the-shelf videoconferencing applications

Use of H323 (or future developing standards ensuring compatibility and interoperability to the existing standards), standards-compliant commercial videoconferencing applications for less demanding tele-collaboration sessions, that allow for live videoconferencing, file transfer, program sharing etc.

2.1 One (1) Scanner One high quality film-scanner suitable for medical applications is required per site, in order to facilitate the digitalization of medical images, x-rays etc.

2.2 One (1) Remote control camera & one (1) Document camera
2.3 One (1) Video projector
2.4 One (1) screen
2.5 One (1) Audio system

With respect to the diversity of the Telemedicine apparatuses and infrastructure available to each SIMIHO the final recommendation consisted of the following:

3.1.1 Each Party is to built / assemble / create a computer infrastructure capable of video-conferencing and complying with the technical specifications described in Table 2, as well as communication infrastructure, shown in Table 3.

3.1.2. Each party is to utilize a single, common to all Parties, provider of satellite interconnection among the hospital computer infrastructures mentioned above, with the characteristics included in Table 4.

3.1.3. In case of a SEDM Country already maintaining a fully operational Telemedicine system, this Country is responsible for establishing an interactive linking facility to the telemedicine network under construction by the other SEDM Countries, providing complete compatibility, interoperability and performance equivalent to the rest of the above telemedicine network.

3.1.4. Each party is to utilize the satellite interconnection facility according to the principles laid down in Table 4;

3.1.5. The cost of creating, modifying, assembling and maintaining the parts of the telemedicine network under construction for each Party is the sole responsibility of the SEDM Country.

Table 4: Description of the characteristics of the satellite interconnection provider for the SEDM telemedicine network.

1. Fully integrated system, capable of providing the following applications**:**

    1.1 Tele-diagnosis
    1.2 Tele-consultation (videoconferencing)
    1.3 Medical tele-training.

2. Satellite facility operating on multi-satellite (satellite network) support (avoiding of “single point failure”) and providing a full coverage of all Parties and Europe.

3. Maximization of image performance with minimization of bandwidth utilization (2Mbps recommended for interactive communication).

4. Effective and efficient utilization of IP protocol over satellite.

5. Possibility for exclusive (“pay-per-use”) utilization of the bandwidth.

6. Billing system.

7. Network management utility.

8. Option for integration with terrestrial networks or other networks.

3.1.6. Additionally, the cost of utilization of the satellite facility will be shared equally among the interconnecting Parties, according to the billing system of the facility, as appears in Table 4.

4. DISCUSSION

With ongoing work, SIMIHO Med WG was delighted to realize that very few compatibility issues existed among hardware and software utilized in the military hospitals located mainly in capital cities of the SEDM Countries. Having given the fact that the concept accepted by the SEDM MODs concerning a satellite facilitated network (Figure 2), is a simple, cost effective and readily deployable in principle network, this WG had to address several issues arising in three separate and distinguishable steps:

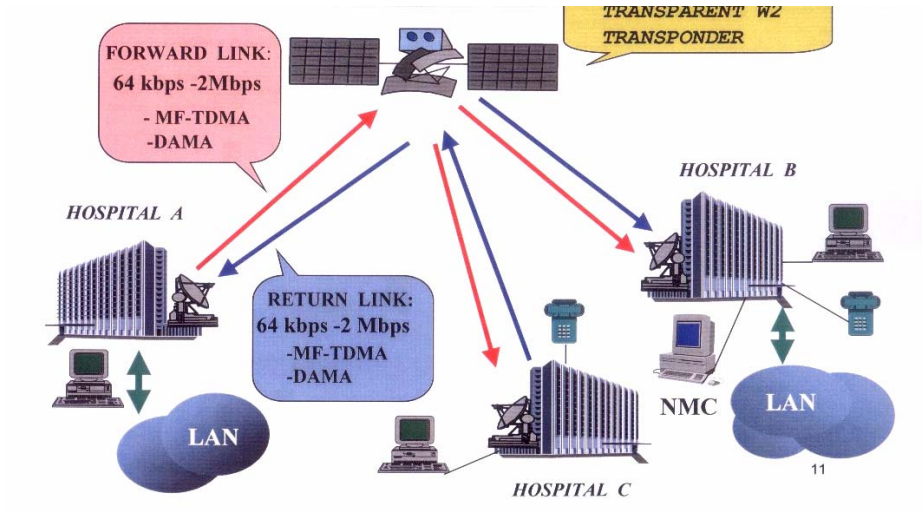

Figure 2: Schematic representation of the concept for a satellite-facilitated medical network

4.1 Address diversity among SEDM Countries

The technological status level of medical surveillance for each SEDM country was different, but may be summarised as shown in Table 5. Of all SEDM Parties, only US and IT operate a similar network; the US system is operationally centered in Landstuhl, GE, while the Italian one in Rome. AL experienced funding problems and CR does not maintain a central military hospital, however, they are in support of implementation of the project. The remaining Partners (BU, FY, GR, RO, TU) will implement SIMIHO.

Table 5: Diversity among SEDM Countries regarding network and ratification / implementation of SIMIHO project.

SEDM Countries	Ratifying and implementing	Ratifying but not implementing
With existing operational satellite network	US	IT
Without an existing operational satellite network	BU, FY, GR, RO, TU	AL, CR

For the Ratifying but not implementing partners, the WG proposed issuing of explicit instructions, guiedelines and technical manuals to enable active participation when their infrastructure becomes available (AL, CR), or become ready to meet the cost of adapting their system to the SIMIHO one (IT).

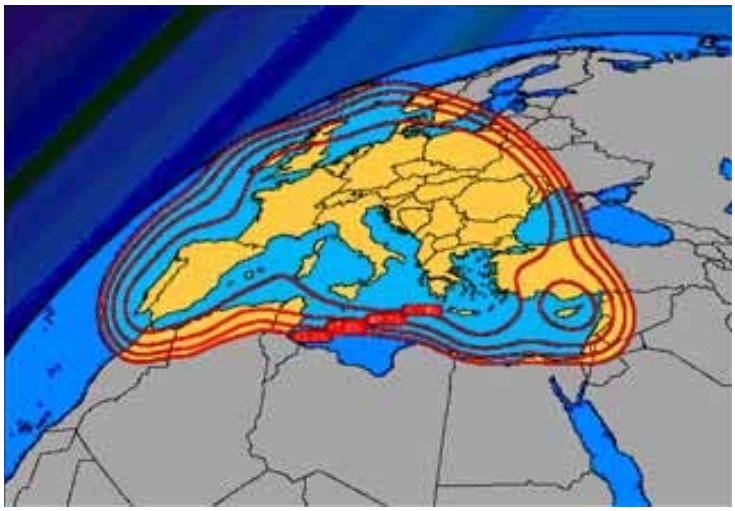

Figure 3: Beam area of Hellas Sat (2003)

Figure 4: Medical surveillance of vital signs in a space mission

4.2 Cost Effectiveness

The requirement for Possibility for a billing system, as laid down in Table 4 together with an exclusive ("pay-per-use") utilization of the bandwidth, provides a minimum service cost, which may be tailored to individual needs. Depending on the satellite service provider, the hourly cost of a 2Mbps bandwidth may well be under 75 Euro, and availability of services covering the Balkan area is not rare (Figure 3).

4.3 Operational Impact

The operational potential of a satellite interconnection network is significant: except for the capacity to promulgate international co-operation and build trust among the SEDM Patrners, a political target, it may well serve in other national and international occasions:

4.3.1 Operational medical surveillance during multi national deployments, such as in SEEBRIG located in Plodviv (Filippoupolis) in BU, and in other theatres woldwide (e.g. The telemedicine system employed by US Regional Medical Command Europe to monitor troops deployed in Balkan theatres (Hill 722 & Camp Corner, Eagle Hospital in Bosnia, Bondsteel Hospital in Kossovo - ERMC/LRMC USAEUR).

4.3.2. Although the network official interconnection point will be the designated military hospital (NATO Role 4), there is no restriction to further develop the network within country limits: each Partner may further expand the network via optical fibres or wireless coupling to include further hospitals or mobile units (ships, isolated units surrounded by desert areas). This may facilitate public and military health systems, either by providing essential medical information pre-, post- or during deployment, or provide a forum for international co-operation in cases of mass casualty/destruction (e.g. earthquakes, forest fires, etc). The latest development in medical surveillance presents remote surveillance of vital signs of VIP personnel, such as the ones in orbit (Figure 4).

4.4. Relationship to other NATO telemedicine activities.

Possible overlap with other Telemedicine initiatives within NATO was also considered, such as the NATO Telemedicine WG (TMED WG). The aim of SIMIHO WG is to provide a satellite interconnection mainly, but not exclusively among military hospitals (operational NATO role 4), presents a high-end specifications application (minimum speed: 2Mbps), with immediate applicability based on an accepted common hardware and software standard. The NATO Telemedicine WG is orientated towards operational roles 1-4, presents a low-end specifications

application (Maximum speed: 0.18 Mbps, the current NATO standard) and is responsible for producing a STANAG, which is subjected to time-consuming national ratification and implementation procedures, also dependent on existing national reservations. The relationship between SIMIHO and TMED remains close, with the SIMIHO/Tech Sectetary updating TMED in their Oslo, NO Sept 2003 meeting, and SIMIHO member commenting on the newly developed TMED STANAG.

5.0 CONCLUSION

The SIMIHO Med WG proposal for a Satellite facilitated Interconnection of the Military Hospitals of the SEDM countries presents a valid, cost-effective, independent of ground texture or environmental associated issues, realistic and cost effective means of serving the SIMIHO purpose.

6.0 References: ..

[1] Joint Statement SEDM Ministers Of Defence/09-10-2000, Thessaloniki, GR

[2] Joint Statement SEDM Ministers Of Defence/21-12-2001, Antalya, TU.

SYMPOSIA DISCUSSION - PAPER 17

Authors Name: Dr Diamantopoloulos (GR)

Discussor's Name: Dr Clere (FR)

Question:
What is the robustness of the network in the case of many thousands of simultaneous users accessing the network in case of crisis, war?

Author's Reply:
A cell phone network is rapidly saturated if a lot of users have to use their cell phones.

For a satellite network, it is not the case because there is a constellation of satellites and, if one satellite is saturated the network transfers the work to the other satellites of the network. Thus, the network provides insurance against saturation which is not present in land-based cell technologies.

Authors Name: Dr Diamantopoulos (GR)

Discussor's Name: Capt (RNL)Hovens (NL)

Question:
Did the committee think of connecting the military hospital "by wire" and hook up the remote installation by satellite?

Author's Reply:
It is politically given circumstance to use satellite communications in both locations.

Research Potential of a Heart Rate Variability Diagnostic System for the Study of Stress and Health Risk in Peacekeeping Operations

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ABSTRACT

Medical surveillance and medical screening for military personnel participating in Peacekeeping Missions (PKM) is required to define the effect of peacekeeping stress on their health status and to determine suspected risk indicators. The aim of the present study is to determine the effect of stress on autonomic cardiovascular control and health risk of 72 Bulgarian peacekeepers participating in PKM in Kosovo. A diagnostic system for the analysis of Heart Rate Variability (HRV) was used for psychophysiological assessment of stress and screening of health risk at peacekeeping deployment phases. Personal interviews were implemented to reveal the nature of the stressors. HRV variables and heart rate were compared between pre-deployment and re-deployment phases and between deployment phases and controls. As a response to cumulative exposure to the effect of stress on cognitive functions, we measured decreases in parasympathetic activity with P_{RSA}, and STV and decreases in baroreceptor modulation of heart rhythm with P_{THM}. Identifying peacekeepers with suspected health risk and differentiating basic types of autonomic control (as a response to level of stress) associated with referent, pre-morbid and morbid states may be an important risk indicator for the assessment of health status. The advantage of psychophysiological assessment of peacekeepers stress response at deployment phase using functional indices of stress and health risk is that it provides objective information about the impact of stress on their health.

1.0 INTRODUCTION

A key topic of military medical science and ethics in the 21st century concerns ensuring optimal health status, optimal physical and psychological fighting efficiency, high expertise and professionalism, to

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consolidate freedom in the world; protect world peace; guarantee defence, safety and security of humanity in the fight against terrorism and tyranny; and to preserve principles and values of the world democratic community.

1.1. The Causes for Organization of Peacekeeping Missions. Nature of Stressors.

One of the main tasks of military medical science is to implement health surveillance, screening and risk assessment of military personnel participating in Multinational Missions, including Peacekeeping Missions (PKM). PKM organize as a response of the consequences of tyranny and terrorism. The traditional role of peacekeepers is to maintain strictly neutral presence by implementing and overseeing peace settlements, monitoring elections, and facilitating the delivery of humanitarian aid [Litz et al, 1997]. But the potential terrorist and security threats placed increased demands on military personnel in the 21st century as the military forces have to safeguard not only the regional areas but also maintain global peace [Masakowski, 2001]. This requirement forms the new identity and the new role of military forces, including peacekeepers in their task to ensure the new security environment in the 21st century.

The UN, NATO, EU, the world democratic community and the new democratic institutions of the 21st century which have had to cope with tyranny have defended the enduring principles of civilization: freedom, progress, pluralism and tolerance without which humanity can not continue developing normally. Tyranny and terrorism and their consequences are the main sources of stress for humanity and a threat to the peaceful and creative life of millions of people wherever they live and work. Terrorism is a stressor without face but it possesses its genetic sources. The cause and force of terrorism is political (fascism; communism and its extreme form bolshevism) and religious (Islamic fundamentalism) fanaticism. Today's variant of violence is provoked from archaic and unacceptable for the 21st century Islamic fundamentalism. But we representatives of the post-communistic countries remember well what was presented by the countries of the Eastern block before 1989. History showed that the consequences of all totalitarian regimes are civil wars, inter-ethnic conflicts, and humanitarian disasters.

The world democratic community has engaged in the fight against tyranny and terrorism and has upheld efforts of NATO and the UN to establish peace in conflict zones through organization of Multinational Missions. NATO and the UN have interceded and have provided peacekeeping operations, protecting civilians who are at risk, providing humanitarian relief, ensuring armed enforcement of peace, and arranging ad hoc coalitions for the maintenance of world peace.

1.2. Stress Exposure in Peacekeeping Missions. Psychological Health Surveillance.

Peacekeeping missions are dangerous and are characterized by conflicts, incidents, and challenges. Therefore the task of the military medicine, which considers the health status and the psychological and physical well-being of military personnel as important indicators of operational effectiveness, is to control medical readiness [Capleton et al, 2003; Cox, 2003]. The medical readiness could be compromised during phases of peacekeeping deployment when personnel are exposed to stress. The task of medical readiness, which is studied and optimized through medical surveillance and medical screening, is to analyze, treat, preserve human health and performance, and ensure mission effectiveness.

A specific requirement for military personnel participating in these operations is the ability to maintain balance between combat readiness and the exercise of restraint [Wright et al, 2002]. Both parts of this definition presuppose that participants are exposed to: the impact of potentially traumatic combat war-zone situations (combat attack and incidents; dangerous patrols; witnessing death and injury; handling bodies), terrorist attacks, and the impact of social and psychological stressors. An extensive theoretical formulation of the stressors involved in peacekeeping is given in the study of Lamerson & Kelloway, 1996 in which they characterize peacekeeping by the occurrence of acute and/or catastrophic events in an environment replete with chronic stressors, and classify peacekeeping stressors as follows: combat stressors (direct exposure to

attack; witnessing death or injury; traumatic nature of handling dead or wounded bodies); chronic stressors (role stressors: conflict, ambiguity, overload, family stress).

A recent study of Breakwell & Spacie, 1997 identifies operations in the Gulf War and Bosnia, develops a typology of stressors dividing them into four principal types: organizational, physical, interpersonal, and psychological. Another study analyzed frustrating and potentially traumatic experiences associated with PKM in Somalia, formulated specific dimensions: positive aspects of military service (visiting a new country), positive aspects of humanitarian missions (bringing food to starving people), low-magnitude stressors (being separated from family), negative aspects of peacekeeping (not being appreciated by the Somalis), and exposure to war-zone stressors (going on patrols) [Litz et al, 1997].

Bartone & Adler, 1998 consider the constant threat of terrorism faced by deployed peacekeeping and contingency forces as one of the strongest combat and psychological stressors. They point out that the unpredictability of such threats on PKM and the contrast they present to regular daily routines when they do occur increase the risk for Post-Traumatic Stress Syndrome (PTSD) or dissociative disorders.

Except for combat stressors, peacekeeping deployment is characterized by exposure to social and psychological stressors and such challenges as helplessness, powerlessness, isolation, boredom, monotony, separation from family, role stressors, economic factors (inadequate or undeveloped infrastructure), nationalistic interests, non-recognition of human rights (ethnic tension), state corruption, organized crime, drug and people trafficking, and provocations [Carlstrom et al, 1990; Harris & Segal, 1985; Litz, 1996; Ritchie et al, 1994; Weisaeth et al, 1996; Weisaeth & Sund, 1982; Wright et al, 2002].

A specific very stressful feature during exposure to peacekeeping stressors is the requirement to exercise restraint which is regarded as a principal and crucial component of effective peacekeeping [Allard, 1995; Moskos, 1975; Wright et al, 2002]. This feature is stressful because it requires to exercise restraint in the case of provocation, danger, threat; it contributes to feelings of helplessness, increased anxiety and frustration [Litz, 1996; Moskos, 1975; Segal & Segal, 1993]; and might induce PTSD or be a mediator of the relationship between PTSD and other stressors [Litz et al, 1997]. To exercise restraint is a very stressful requirement for peacekeepers from democratic societies. Even the psyche and the physical condition of very trained and healthy participants may not endure the effects of stressors – consequences of political and religious fanaticism: genocide, atrocities, and aggression. Their psyche can not withstand the encroachment of human freedom as the participants in PKM come from democratic societies not accustomed to the repressive mechanism and structures of totalitarian regimes. The morale and psyche of military personnel during contemporary peacekeeping deployment are seriously affected by the dangerous humanitarian missions (witnessing violence, receiving hostile response from the civilian population, witnessing human degradation) [Dirkzwager et al, 2003]. The cognitive load on their psyche increases when their norms contradict with the mentality and consequences of repressive totalitarian regimes: encroachment on human being and freedom (genocide and atrocities); non-recognition of human rights; repressions; and provocations by nationalistic parties and movements. Military medical studies on combat stress during World War II, the Holocaust, Vietnam and the Gulf War reveal the effects of the same stressors and moral conflicts that mediate PTSD [Bergherr et al, 1997; Bramsen & Van Der Ploeg, 1999; Friedman et al, 1994; Green et al, 1990; King et al, 1995; Kuch & Cox, 1992; Williams et al, 1993].

The operational environment of the PKM is characterized by different chronic stressors that act separately or together and that can result in stress which affects health status and degrades performance. The nature of contemporary PKM has changed compared to previous traditional missions as conflict situations and incidents are increased. Analysis of the changing nature of contemporary PKM reveals that peacekeeping deployment comprises exposure to traumatic stressors in a context of chronic stressors (Lamerson & Kelloway, 1996). The analysis shows also that the simultaneous experience of traumatic and chronic stressors may have replicative rather than additive or specific effects [Kessler et al, 1995; Resnick et al,

1993]. Thus, both single and cumulative (repeated) exposure to potentially traumatic events may evoke symptoms consistent with a significant stress response in PKM [Bolton et al, 2001; Charney et al, 1993].

Participants are exposed to different kinds of stressful experience that may put them, independently of their good health status and high level of psychological and physical selection and training, at risk for development of medical and psychological problems (PTSD), Peacekeeper Stress Syndrome, mental health symptoms. Health services are needed for military personnel participating in PKM, as during the deployment phases the personnel may develop psychological disorders as a consequence of participation in PKM. In this component of health surveillance, psychological screening is used to determine risk indicators and to predict morbid outcomes, pre-deployment psychological issues, re-deployment acute stress reactions, and post-deployment psychological adjustment [Wright et al, 2002]. The results of Dutch investigators indicate that social support and coping strategies may be valuable aspects for preventing PTSD [Dirkzwager et al, 2003]. In a study investigating mental and physical health, pre-deployment phase and the six-month follow-up were related to higher levels of anxiety, psychological stress, depression, and somatic symptoms compared to other phases [McDonald et al, 1998]. Traditional combat and negative aspects of peacekeeping mission in Somalia led to PTSD and frustration [Litz et al, 1997]. Peacekeepers stress exposure in PKM in former Yugoslavia correlates significantly with depression and psychiatric symptoms [Bartone & Adler, 1998], and the psychological dimensions studied: isolation, ambiguity, powerlessness, boredom and danger were found to be relevant to adaptation to stress. Results from Norwegian researchers showed that PKM is associated with PTSD, anxiety, health problems, and suicidal behavior [Weisaeth et al, 1996]. Japanese researchers reveal that deployed personnel show somatization symptoms [Kodama et al, 2000]. Other studies assess the stress tolerance and the health condition of military personnel as generally good with low prevalence of PTSD [Hotopf et al, 2000; Johansson et al, 2003; Ponteva, 2000; Ponteva et al, 2000].

1.3. Psychophysiological Assessment of Autonomic Responses to Stress in Post-Traumatic Stress Disorder

We give a short review of the autonomic responses to stress in combat veterans and survivors from the Holocaust with PTSD as we believe it will contribute to our understanding of the autonomic responses to stress in PKM. Despite the increasing number of studies investigating the nature of stressors in PKM, their consequences for psychological health and the intervention strategies for their prevention, at this time in military medicine there is little research on the effects of stressful peacekeeping deployment on functional state assessed by means of physiological and psychophysiological measures. The examination of functional state with psychophysiological indices in different deployment phases will contribute to: the assessment of objective health status of peacekeepers participating in PKM, to medical surveillance, and to the screening of stress and health risk as well as treatment and prevention.

In relating autonomic responses to peacekeeping stress with the autonomic responses to PTSD, we consider the following issues: exposure to traumatic and chronic stressors inducing stress reaction; autonomic responsivity and arousal in response to stress; elevation in cardiovascular activity and cardiovascular function; role of psychophysiological assessment in investigation of autonomic arousal and reactivity; chronic (repeated) autonomic responses, and cardiovascular reactivity. These processes may induce structural and/or functional disturbances, and PTSD might be a risk factor for CVD.

Considerable research has been implemented on the causes, pathophysiological mechanisms, and symptoms of PTSD. In our study we are focusing our research interest on PTSD resulting from exposure to combat stress during World War II, the Holocaust, and the Vietnam War. The nature of these traumatic events is comparable to the potentially traumatic combat situations that induce peacekeeping stress.

PTSD is a psychological illness of considerable prevalence, treatment resistance, and chronic course. Specific symptoms of PTSD are persistent reexperiencing of the traumatic event, avoidance of stimuli

associated with the trauma, and autonomic hyperarousal [Charney et al, 1993]. Indicators of increased arousal characterizing PTSD are: hyperalertness, exaggerated startle response, and increased physiological reactivity to stimuli that symbolize or resemble the traumatic event [Buckley & Kaloupek, 2001; Cohen et al, 1997; Kaloupek & Bremner, 1996; Liberzon et al, 1999; McFall et al, 1990]. The major clinical symptoms of PTSD are: autonomic disturbance, tachycardia, increased blood pressure, tachypnea, tremor, and excessive sweating [Kolb, 1987].

Psychophysiological measures are used in the study of functional mechanisms inducing autonomic arousal and physiological reactivity [Blanchard & Buckley, 1999; Buckley & Kaloupek, 2001; Cohen et al, 1997; Liberzon et al, 1999; McFall et al, 1990]. Results of psychophysiological studies on PTSD have shown that the most relevant cardiovascular measures are: heart rate, heart rate variability, blood pressure, systolic and diastolic blood pressure variability.

Different research views and hypotheses exist for the explanation of autonomic hyperarousal and increased physiological reactivity. The observed patterns of activity of autonomic cardiovascular control might be the following: increased sympathetic activity [Blanchard et al, 1991; Bremner et al, 1999; Keane et al, 1985; Kolb, 1987; Kolb, 1984]; and increased sympathetic activity and suppression of parasympathetic activity [Cohen et al, 1997; McFall et al, 1992]. Kaloupek & Bremner, 1996 suggest that in psychophysiological studies of PTSD we should explore the model of autonomic activation of Berntson et al, 1991, which includes sympathetic activation; parasympathetic withdrawal; concurrent activation in both branches with the accent on sympathetic dominance.

In research of PTSD we should also take into consideration whether traumatic exposure affects the tonic level of autonomic activity or phasic changes as reflected in the autonomically controlled functional response measures. Some of the results in this respect suggest that PTSD is characterized by elevated tonic level of sympathetic activity at rest reflected in increased heart rate, blood pressure, and increased low frequency component of heart rate variability [Blanchard, 1990; Buckley & Kaloupek, 2001; Cohen et al, 1997; Liberzon et al, 1999; Pallmeyer et al, 1986]. Other results show phasic activation of sympathetic activity in response to traumatogenic stimuli [Kaufman et al, 2002; Malloy et al, 1983; McFall, 1990; Pallmeyer et al, 1986]. Cohen et al., 1997 point out that both increases of sympathetic activity and decreases of parasympathetic activity (studied with spectral measures of heart rate variability) contribute to the changes in tonic level of autonomic activity at rest which induce autonomic hyperarousal. Contrary to these results some studies report no difference in tonic level of sympathetic activity at rest comparing PTSD and control groups [Malloy et al, 1983; McFall et al, 1992]. Phasic changes of autonomic activity to traumatogenic stimuli are characterized by activation of sympathetic function and suppression of parasympathetic activity [Blanchard et al, 1991; McFall et al, 1992]. Structural and functional changes in the cardiovascular system (increased peripheral vascular resistance and increased blood pressure) observed in PTSD are thought to be a result of chronic stress-related sympathetic activation and disturbed regulation of beta-adrenergic receptors [Amerena & Julius, 1995; Buckley & Kaloupek, 2001; Hocking-Schuler & O'Brien, 1997].

Considering that:

- The effect of stress on functional and psychological condition is the cause to be implemented in medical surveillance of health status,
- The screening of health status as an important component of medical surveillance has been extensively used to determine risk indicators and to predict morbid states,
- Psychophysiological assessment of autonomic responses in PTSD,
- The identical nature of combat stressors,

our study is an attempt to clarify whether peacekeeping stress affects the functional status of cardiovascular system and the underlying autonomic cardiovascular control. The screening of health status in deployment phases will help us to determine risk indicators and to prevent development of morbid states. Medical surveillance of the health status of peacekeepers is implemented by the research and technology opportunities of medical informatics technology. We explored opportunities of the diagnostic system for

medical surveillance and risk assessment: Heart Rate Variability for determination of stress response and deviation in autonomic regulation as an early indicator of health risk in peacekeepers participating in PKM.

The aim of the present study was to determine the effect of stress on autonomic cardiovascular control and health risk of peacekeepers participating in PKM in Kosovo.

2.0 METHODS

2.1. Subjects

Two groups of subjects participated in this study: military personnel and controls. The first group consisted of 72 male peacekeepers who are members of the Bulgarian armed forces whose ages ranged from 20 to 43 years (mean age, X±SD, 28.34±10.07 yr). They were deployed on a six month peacekeeping mission in Kosovo, which was the first PKM mission they participated in. The rank of 69 individuals was soldiers and sergeants, and 3 were commissioned officers. They were selected to be physically and psychologically healthy and suited for deployment according to NATO standards. The study was longitudinal for the military personnel group. The peacekeepers were examined in pre-deployment and re-deployment phases. The control group consisted of 61 male individuals who were employees in institutions matched for age (mean age, X±SD, 28.12±9.31 yr) to the peacekeepers. The control group is used for the first investigation of the experimental group. The following exclusion criteria were used to both groups: systolic blood pressure >130 mmHg and diastolic blood pressure >85 mmHg; body-mass index >25kg/m^2; using of medications, alcohol, nicotine, caffeine; history of diseases.

2.2. Procedure

2.2.1. Computerized diagnostic system for analysis of Heart Rate Variability

A computerized diagnostic system for medical surveillance of functional status of cardiovascular system (CVS): Heart Rate Variability (HRV) was applied. The system consists of: PC-IBM, specialized hardware and software [Danev, 1989; Nikolova, 1993] that enables the following tests for the assessment of functional state of the CVS: Cardiotachogram; Histogram; Scattergram; Power Spectrum Analysis of HRV, Mental and Physical Stress, Health Risk.

HRV data were determined from ten minutes of ECG recordings between 9 a.m. and 11 a.m. in supine position after a one-hour rest period. HRV data were obtained on three consecutive days and mean individual values of the measurements were calculated.

A portable electronic device was used to transform the ECG signal into RR intervals and to transmit the RR intervals to an IBM compatible PC for on-line processing. The ECG signal was transformed to RR intervals with an AC converter (QRS detector and timer, resolution time 2224 samples per second). This sampling rate gives a variation of 0.48 msec in locating the peak of R wave and results in a minimum accuracy of 99.55 % in computing heart rate up to 140 beats/min.

Time-domain and frequency-domain based HRV measures and HRV derived indices were analyzed:

2.2.1.1. Time-domain HRV measures:

X (mean RR interval) (msec), resp. mean heart rate (beats/min); Short-Term Variability (STV) (msec) (reflecting respiratory oscillations in heart rate variations); Long- Term Variability (LTV) (msec) (reflecting baroreceptory- and thermoregulatory influences on heart rate variations); Time-Domain Index (TDI) (arb. un.) (assessing sympathetic/parasympathetic influences on histogram RR interval distribution).

2.2.1.2. Frequency-domain HRV measures:

Spectral power of RR intervals in the Temperature band (0.01-0.05 Hz) (P_T) (ms^2) (sympathetically mediated); Spectral power of RR intervals in the Traube-Hering-Mayer band (0.06-0.14 Hz) (P_{THM}) (sympathetically and parasympathetically mediated) (ms^2); Spectral power of RR intervals in the Respiratory Sinus Arrhythmia band (RSA) (0.15-0.50 Hz) (P_{RSA}) (ms^2) (parasympathetically mediated); Frequency-Domain Index (FDI) (P_T/P_{RSA}) (arb.un) (reflecting sympathetic/parasympathetic activity ratio). Spectral powers of RR intervals in the respective frequency bands were calculated using Fast Fourier Transform.

2.2.1.3. HRV-derived indices:

Physical Stress (PS) (arb. un.) (mathematical algorithm based on difference between measured and age-referent values derived from the time-domain HRV measures); Mental Stress (MS) (arb. un.) (mathematical algorithm based on difference between measured and age-referent values derived from the frequency-domain HRV measures); Functional Age (FA) (yr) (mathematical algorithm computing difference between measured and age-referent values of autonomic activity derived from the frequency-domain HRV measures); Health Risk (HR) (%) (mathematical algorithm derived from PS-, MS-coefficients and number of premature heart beats).

2.2.2. Computerized Method for Detection of Supraventricular and/or Ventricular Extrasystoles

The determination of extrasytole type was done by using a computer method [Danev, 1989] for detecting supraventricular and ventricular extrasystoles in HRV recordings.

2.2.3. Personal Interviews

Personal Interviews with peacekeepers were implemented to examine stressors. Results of interviews showed that peacekeepers were exposed to the following types of stressors: risk of terrorism; potentially traumatic combat situations (incidents of combat attacks; dangerous patrols; safeguarding of important objectives; increased risk of escalating conflicts); social and psychological stressors (deployment in new environment; provocations; work on duties; consideration with the way of life, customs, and religion of the native population; separation from family; limited access to civilian places).

2.3. Data Analysis

HRV measures, HRV-derived indices and heart rate in peacekeepers and control groups are expressed as means ± standard deviations. Means of HRV variables were compared by paired samples t-test and independent samples t-test. The differences between the mean values of HRV variables and heart rate among military personnel group at the re-deployment phase diagnosed with referent, pre-abnormal and abnormal autonomic cardiovascular control were calculated by independent samples t-test. The group was divided in terms of different types of autonomic cardiovascular control based on referent values of HR. The division was done to determine the association between abnormality in autonomic cardiovascular control and suspected pre-morbid and morbid states. Discriminant analysis was used to define which measures distinguish basic types of autonomic cardiovascular control in the re-deployment phase. A p value < 0.05 was considered statistically significant.

3.0 RESULTS

To examine whether there are differences in level of stress exposure between deployment phases in military personnel and how they differ from controls, HRV variables and heart rate were compared between each condition by t-test. The mean values of HRV variables and heart rate in peacekeeper deployment phases and controls are presented in Table 1.

Table 1: Means (X±SD) and p – values of time- and frequency – domain HRV measures, HRV – derived indices and heart rate in pre – deployment and re – deployment phases and in control group.

Variables	*Pre-deployment phase - 1*	*Re-deployment phase - 2*	*Control group - 3*	*P - value*		
	X±SD	*X±SD*	*X±SD*	*1-2*	*1-3*	*2-3*
Heart rate (b/min)	76.5 ± 11.22	75.30± 9,32	72.31±10.35	ns	ns	ns
Time-domain HRV measures						
X (msec)	800.53±118.13	769.89±111.78	846.18±121.87	ns	ns	ns
STV (msec)	68.03±7.655	52.81±6.50	67.03±6.38	0.01	ns	0.01
LTV (msec)	48.39±7.56	43.53±7.34	45.21±7.34	ns	ns	ns
Frequency-domain HRV measures						
P_T (ms^2)	9.80±1.00	8.13±1.64	10.15±1.82	ns	0.02	<0.0001
P_{THM} (ms^2)	12.63±1.84	9.42±1.33	12.00±1.55	0.007	ns	0.03
P_{RSA} (ms^2)	11.80±1.65	6.58±1.56	12.86±1.38	<0.0001	ns	<0.0001
HRV – derived indices						
HR (%)	23.94±6.15	50,78±7,73	25.03±6.76	<0.0001	ns	<0.0001
PS (arb. un.)	-0.34±0.06	0.92±0.05	-0.12±0.03	0.007	0.5	<0.001
MS (arb. un.)	0.40±0.09	1.03±0.09	0.26±0.05	0.001	ns	<0.0001

Figure 1: Mean values of STV (msec) in peacekeepers: pre-deployment and re-deployment phases and in control group

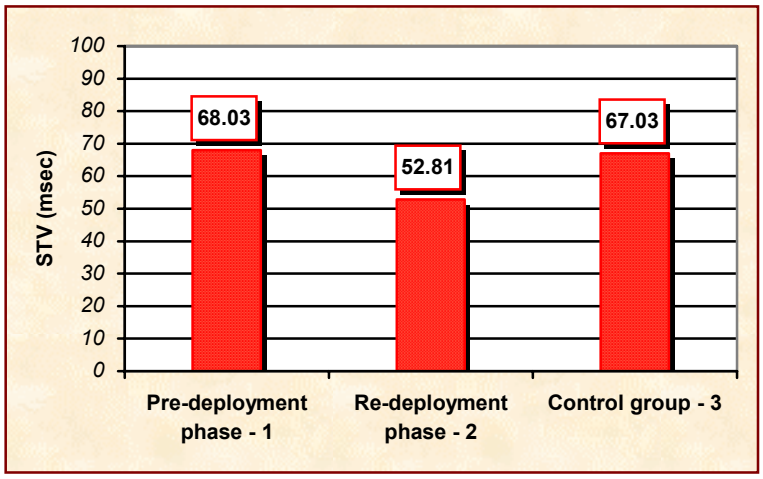


Figure 2: Mean values of P_T (ms^2), P_{THM} (ms^2) and P_{RSA} (ms^2) in peacekeepers: pre-deployment and re-deployment phases and in control group

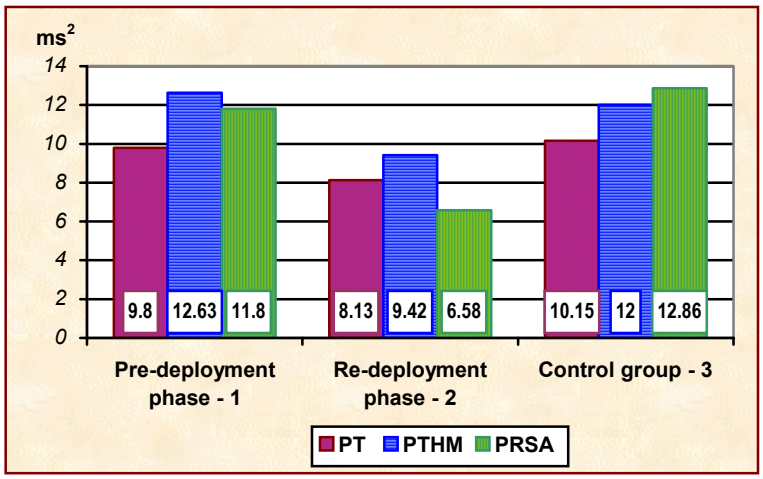


Figure 3: Mean values of HR (%) in peacekeepers: pre-deployment and re-deployment phases and in control group

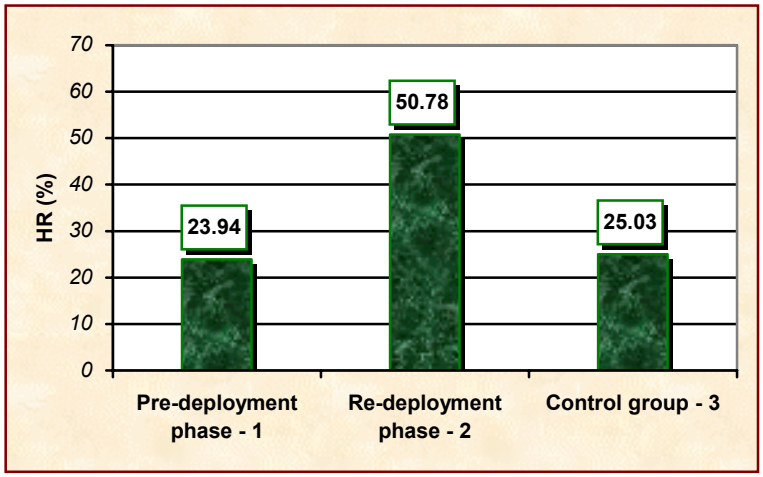
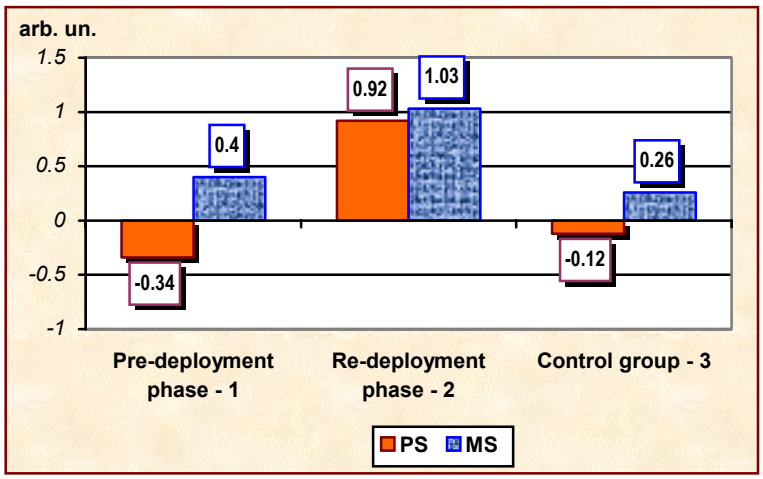


Figure 4: Mean values of PS (arb. un) and MS (arb. un.) in peacekeepers: pre-deployment and re-deployment phases and in control group



Stress was associated with a significant decrease in mean values of STV, P_{THM} and P_{RSA} in peacekeepers in the re-deployment phase compared to the pre-deployment phase. Peacekeeper stress resulted also in a significant increase in mean values of HR, PS, and MS in the re-deployment phase compared to the pre-deployment phase. Fig. 1, fig. 2, fig. 3 and fig. 4 illustrate differences in mean values of STV, P_{THM}, P_{RSA}, HR, PS, and MS in peacekeepers in the two deployment phases. The results also revealed that mean values of STV, P_{THM}, P_{RSA}, HR, PS, and MS differed significantly when comparing peacekeepers in the re-deployment phase with the controls (Fig. 1-4). The mean values of heart rate did not show significant differences in both peacekeeping deployment phases and between peacekeepers and controls.
We did not detect supraventricular or ventricular extrasystoles in peacekeepers and controls.

Although HR was in the range of referent values it was significantly higher in the re-deployment compared to pre-deployment phase (Table 1). An increase of HR value above 65% is an indicator for the development of CVD [Danev, 1989]. To examine whether the re-deployment phase may be differentiatiated as a response to level of stress in different types of autonomic cardiovascular control, we believe it is justified to determine whether they are associated with pre-morbid and morbid states. For this purpose we divided the peacekeeper group studied in the re-deployment phase on the basis of referent values of HR, forming two types of autonomic cardiovascular control: referent (N61) and pre-abnormal (N11). We did not observe individuals with abnormal autonomic cardiovascular control. To examine differences in autonomic cardiovascular control and to determine the pattern of autonomic function, HRV variables and heart rate were compared by independent samples t-test. Mean values of HRV variables and heart rate in groups with referent and pre-abnormal autonomic cardiovascular control are presented in Table 2.

Table 2: Means (X±SD) and p – values of time- and frequency – domain HRV measures and heart rate at peacekeepers groups with referent, pre – abnormal and abnormal autonomic cardiovascular control.

Variables	*Types of autonomic cardiovascular control*			*P - value*		
	Referent (1) X±SD	*Pre-abnormal (2) X±SD*	*Abnormal (3) X±SD*	*1-2*	*1-3*	*2-3*
Heart rate (b/min)	74.15 ± 10.71	77.10± 11.49	-	ns	-	-
Time – domain HRV measures						
X (msec)	789.36±110.15	749.53±105.09	-	ns	-	-
STV (msec)	56.73±6.04	48.23±5.18	-	0.01	-	-
LTV (msec)	48.93±7.04	37.33±6.71	-	0.01	-	-
Frequency –domain HRV measures						
P_T (ms^2)	8.97±1.04	7.85±0.86	-	0.05	-	-
P_{THM} (ms^2)	10.31±1.41	8.45±1.05	-	0.001	-	-
P_{RSA} (ms^2)	7.85±1.63	5.32±1.47	-	0.001	-	-

Figure 5: Mean vaues of STV (msec) and LTV (msec) in peacekeepers at re-deployment phase with referent and pre-abnormal autonomic cardiovascular control

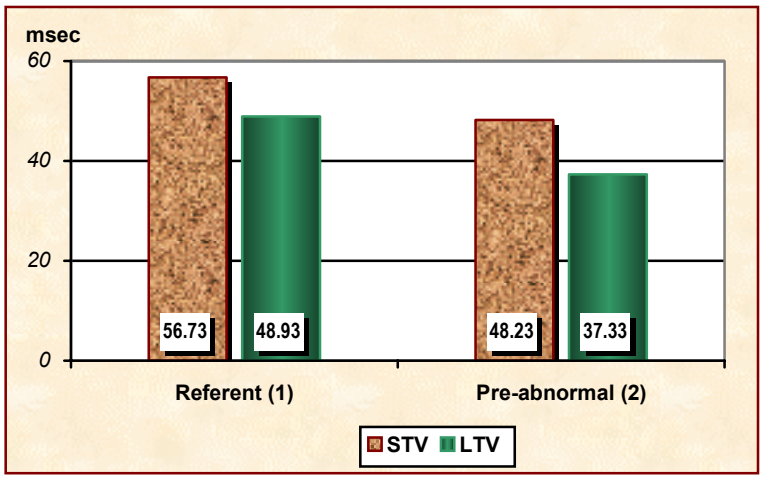
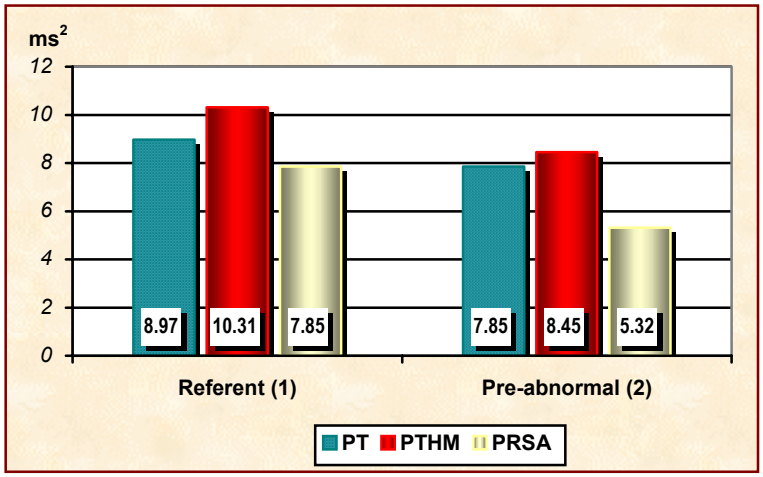


Figure 6: Mean vaues of P_T (ms^2), P_{THM} (ms^2) and P_{RSA} (ms^2) in peacekeepers at re-deployment phase with referent and pre-abnormal autonomic cardiovascular control



Time- (STV, LTV) and frequency-domain (P_T, P_{THM}, P_{RSA}) HRV measures significantly decreased in the peacekeepers with pre-abnormal autonomic cardiovascular control compared to peacekeepers with referent autonomic cardiovascular control. Mean values of heart rate did not show significant differences. Fig. 5 and fig. 6 illustrate differences in mean values of: STV, LTV, P_T, P_{THM}, and P_{RSA} in peacekeepers with referent and pre-abnormal autonomic control.

Results of discriminant analysis revealed that variables that discriminate the referent and pre-abnormal types of autonomic cardiovascular control using HR as a stressogenic marker are: P_{THM}, P_{RSA} , and MS. Results of discriminant function analysis are presented in Table 3.

Table 3. Discriminant function analysis

Clasifficator	*Groups*	*Discriminant Function*	*Percent of Correct Classification*
HR	1. HR < 25 %	HR = -77.31 + 0.12*P_{THM} + 0. 34*P_{RSA} + 0.76*MS	92.7 %
	2. HR 25 – 65 %	HR = -112..3 + 0.19*P_{THM} + 1.23*P_{RSA} + 0.95*MS	87.6 %

4.0 DISCUSSION

A diagnostic system for the analysis of HRV was used in the present longitudinal study for the psycho-physiological assessment of stress and screening of health risk at peacekeeping deployment phases. HRV analysis was applied to study autonomic function as it measures reflect sympathetic and parasympathetic activity [Akselrod et al, 1981; Kitney, 1975; Porges, 1986]. HRV measures are among the most frequently used cardiovascular parameters for studying stress and workload in complex task environment [Hockey et al, 2003; Backs & Boucsein, 2000]. The health screening performed with non-invasive methods such as HRV enables the diagnosis of asymptomatic forms of Coronary Artery Disease and borderline hypertension [Stein et al, 1995].

The results of our study revealed that the autonomic cardiovascular control examined by HRV measures and HRV-derived indices is affected by peacekeeping stressors. The advantage of our study is that it enabled the longitudinal psychophysiological assessment of stress response and the screening of health risk of peacekeepers' health status in deployment phases. Both time- (STV) and frequency-domain (P_{THM}, P_{RSA}) HRV measures, and HRV-derived indices (HR, PS, MS) were changed as a function of peacekeeping stressors. In re-deployment compared to pre-deployment phase we observed a decrease in the mean values of P_{RSA} and STV, which is assumed to reflect Respiratory Sinus Arrhythmia and P_{THM} related to the baroreceptor modulation of heart rhythm. These results indicate a reduction of parasympathetic function and baroreceptor modulation of heart rhythm in the re-deployment phase. The control group is used for comparison with the second experimental period as it is considered that for this period of time significant changes in the control group can not occur.

Psychophysiological assessment of peacekeeper stress response at deployment phases with functional indices contributes to objective information about their health status resulting from the impact of stress. In this regard, the assessment of deployment phases (pre-deployment and re-deployment) and their comparison provide useful information for our further studies: research on pre-deployment phase might provide referent functional data before exposure to peacekeeping stress; the study of the re-deployment phase might provide functional data indicating the effects of cumulative stress. This result is consistent to a certain degree with the study of Wright et al. 2002 who examined health surveillance of peacekeepers in different deployment phases with psychological methods for screening of PTSD and depression.

The most likely mechanism for the observed functional changes in autonomic cardiovascular control is the cumulative effect of: risk of terrorist attacks, potentially traumatic combat stressors, and social and psychological stressors on cognitive functions. The results on the effect of peacekeeping stress on autonomic cardiovascular control are supported subjectively by personal interviews that reveal exposure to the simultaneous effect of risk of terrorist attacks, potentially traumatic combat stressors, and social and psychological stress. Cognitive functions are also affected by the cumulative influence of risk of terrorism, traumatic, social and psychological stressors characterizing PKM. The decrease of vagally mediated P_{RSA} and STV indicating a decline of parasympathetic activity and a decrease of baroreceptor modulation of heart rhythm in re-deployment phase might be affected by the impact of cognitive functions. Involved in these processes is a complex interaction between personal evaluation of a situation and how the individual might change the situation and accomplish successfully his mission. This result is consistent with the results of Shapiro & Katkin, 1980 who indicated that cognitive processes have an influence on physiological functioning and with the results of Wilson, 2003 who revealed that changes in cardiovascular indices are associated with the cognitive load involved in assessing the situation and taking action. The role of cognitive appraisal in the process of interaction between person and environment is pointed out as a mechanism of peacekeeping stress in several studies [Bramsen et al, 2000; Lamerson & Kelloway, 1996].

Besides the psychophysiological assessment of stress, the diagnostic system for analysis of Heart Rate Variability, enables the longitudinal implementation of screening of health risk. In our study the pattern of autonomic changes was associated with increased HR. Although the increase of HR in the re-deployment

phase was 50 % and did not reach the critical value of 65 % for development of CVD, this change is an early indicator for risk of development of pre-morbid state. As a response to level of stress at the re-deployment phase two basic types of autonomic cardiovascular control were differentiated on the basis of referent values of HR: referent and pre-abnormal. Time- and frequency-domain HRV measures decreased significantly in the pre-abnormal compared to referent autonomic cardiovascular control. Pre-abnormal autonomic cardiovascular control might be associated with suspected pre-morbid states. The variables: P_{THM}, P_{RSA} , and MS discriminated significantly the pattern of autonomic function. Results of our occupational studies on the association of basic types of autonomic cardiovascular control (assessed by HRV) with non-specific morbidity revealed significant correlations between abnormal autonomic cardiovascular control and diseases which include in their etiology autonomic dysfunction (e.g., diabetes, hypertension, neuroses, ulcer duodeni, and autonomic disturbances) [Nikolova et al, 2003].

These changes indicate the effects of exposure to risk of terrorist attacks, potentially traumatic combat stressors, social and psychological stressors characterizing PKM in Kosovo and the cumulative effect of peacekeeping stressors on autonomic function and health risk. Our opinion is that most peacekeepers returned from mission with feeling of subjectively experienced stress, because the peacekeepers are young individuals. They are representatives of the new Bulgarian generation and as all normally democratically thinking people who participate in PKM, when they fall into an environment radically different from our country: former Yugoslavia – Kosovo, they react with stress to the consequences of a communistic regime degenerated in bloody nationalistic collisions, ethnic and religious intolerance, terror and violence. The present young Bulgarian generation, with respect to the defence of the freedom and human rights, remind us of another Bulgarian generation which in 1942 - 1943 saved 50, 000 Bulgarian Jews from Nazi concentration camps. This generation had not allowed repressions to be performed on these children, women and men.

The results of our study indicate that the peacekeepers did not show a trend for development of PTSD. We did not observe increasing activity of sympathetic function and its tonic level in deployment phases. Neither heart rate increased nor did the sympathetically mediated time- and frequency-domain HRV measures change as a function of stress. In this respect the testing of the hypothesis to associate the autonomic responses to peacekeeping stress with the autonomic responses to PTSD after exposure to combat stress indicate that the observed autonomic responses are a sign of development of stress reaction and not of PTSD-related autonomic hyperarousal. As a response to cumulative exposure to stress, parasympathetic activity and baroreceptor modulation of heart rhythm are reduced. Identifying peacekeepers with health risk and differentiating basic types of autonomic control (as a response to level of stress) associated with referent, pre-morbid and morbid states might be an important means of assessing health status. The only features that relate peacekeeping stress and PTSD are: exposure to identical traumatic combat stressors inducing stress reaction; role of psychophysiological assessment in stress investigation; stress that might be a risk factor for CVD.

5.0 CONCLUSIONS

In conclusion the results of our study demonstrated:

5.1. Tasks of medical surveillance and medical screening

Medical surveillance and medical screening for the Bulgarian military personnel participating in Peacekeeping Missions is required to define the effect of peacekeeping stress on their health status and to determine suspected risk indicators.

5.2. Research potential of Heart Rate Variability diagnostic system

A diagnostic system for the analysis of Heart Rate Variability might be useful for the psychophysiological assessment of stress and screening of health risk at peacekeeping deployment phases. The advantage of

psychophysiological assessment of peacekeepers stress response at deployment phase using functional indices of stress and health risk is that it provides objective information about the impact of stress on their health.

5.3. Stress and health risk results

These changes indicate the effects of exposure to risk of terrorist attacks, potentially traumatic combat stressors, social and psychological stressors characterizing PKM in Kosovo and the cumulative effect of peacekeeping stressors on autonomic function and health risk.

5.3.1. Stress response

As a response to the effects of cumulative exposure to risk of terrorist attacks, potentially traumatic combat stressors, and social and psychological stress on cognitive functions, we observed reduced parasympathetic activity, examined with P_{RSA}, and STV, and reduced baroreceptor modulation of heart rhythm, examined with P_{THM}. This result is consistent with the results of Shapiro & Katkin, 1980 who indicated that cognitive processes have an influence on physiological functioning and with the results of Wilson, 2003 who revealed that changes in cardiovascular indices are caused by the cognitive load involved in assessing the situation and taking action. The role of the cognitive appraisal in the process of interaction between person and environment is pointed out as a mechanism of peacekeeping stress in several studies [Bramsen et al, 2000; Lamerson & Kelloway, 1996].

5.3.2. Health Risk detection. Screening basic types of autonomic cardiovascular control

Detecting peacekeepers with suspected health risk and diffetentiating basic types of autonomic control: referent, pre-abnormal and abnormal (as a response to level of stress) associated with referent, pre-morbid and morbid states might be an important means of assessing health status. The variables: P_{THM}, P_{RSA} , and MS discriminated significantly the pattern of autonomic function.

5.3.3. Assessment of stress and health risk at deployment phases

The assessment of deployment phases (pre-deployment and re-deployment) and their comparison provide useful information for our further studies: research on pre-deployment phase might provide referent functional data before exposure to peacekeeping stress; the study of the re-deployment phase might provide functional data indicating the effects of cumulative stress. This result is consistent to a certain degree with the study of Wright et al., 2002 as they examine health surveillance of peacekeepers in different deployment phases with psychological methods for screening of PTSD and depression.

5.4. Peacekeeping stress and PTSD

The testing of the hypothesis to associate the autonomic responses to peacekeeping stress with the autonomic responses to PTSD after exposure to combat stress indicates that the observed autonomic responses are a sign of development of stress reaction and not of PTSD-related autonomic hyperarousal. The only features that relate peacekeeping stress and PTSD are: exposure to identical traumatic combat stressors inducing stress reaction; role of psychophysiological assessment in stress investigation; stress that might be a risk factor for CVD.

5.5. Bulgarian peacekeepers – representatives of the new Bulgarian generation

The peacekeepers returned from mission with feeling of subjectively experienced stress. Peacekeepers are young individuals – representatives of the new Bulgarian generation and as all normally democratically thinking people who participate in PKM, when they fall into an environment radically different from our country: former Yugoslavia - Kosovo, they react with stress to the consequences of a communistic regime degenerated in bloody nationalistic collisions, ethnic and religious intolerance, terror and violence. The present young Bulgarian generation with respect to the defence of the freedom and human rights remind us of another Bulgarian generation which in 1942 - 1943 saved 50, 000 Bulgarian Jews from Nazi concentration camps. This generation had not allowed repressions to be performed on these children, women and men.

6.0 ACKNOWLEDGEMENT

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SYMPOSIA DISCUSSION - PAPER 18

Authors Name: Dr Nikolova (BUL)

Discussor's Name: Prof. Dr von Restorff (GE)

Question:
1) Do you have an idea about the variability of your parameters?
2) How much do they change from 1 week after deployment to 1 month after deployment to half a year?

Author's Reply:
1) We have measured the heart rate variability indices in three consecutive days and have taken for the analyses the individual value of the respective heart rate variability measures and heart rate, variability – derived indices.
2) This is our first study on investigating the effect of peacekeeping stress on functional state of the cardiovascular system. We will continue examining and assessing the stability of heart rate variability indices in determining the effect of peacekeeping stressors on the autonomic cardiovascular control and health risk of peacekeepers participating on peacekeeping missions - Kosovo, Iraq, Bosnia, Afghanistan. Results of our previous studies showed that heart rate variability parameters are stable indicators for the assessment of chronic stress and heath risk.

Authors Name: Dr Nikolova (BUL)

Discussor's Name: Capt (USN) Campbell (US)

Question:
1) Assuming that you can overcome the practical, logistics challenges of taking your measurements in real-time, in an operational setting, do you see future applications of such data in predicting functional effects on cognitive features such as state of attention, or spatial/temporal memory?
2) In other words, do you see this type of measurement being of any real value or impact in helping commanders better understand the cognitive "state" of their troops?

Author's Reply:
1) The future applications of autonomic cardiovascular indices in predicting the effect of peacekeeping stress on cognitive functions will continue. We will continue examining the effect of peacekeeping stressors and the corresponding changes in cognitive functions on cardiovascular functional state and the underlying autonomic mechanisms. With our colleagues we will recommend to Commanders and the Military staff the real value of autonomic cardiovascular indices for the assessment of the effect of peacekeeping stressors on cognitive functions and mechanisms.

Authors Name: Dr Nikolova (BUL)

Discussor's Name: Dr Jett (US)

Question:
1) How many months was peace keepers assigned to their post?
2) Did you assess immune state before/after the assignment?

Author's Reply:
1) 6 months
2) No

Biotechnology for Near Real-Time Predictive Toxicology for Warfighter Protection

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ABSTRACT

An increasingly important issue in force protection is the toxicology associated with toxic chemical and mixture exposure at uncharacterized deployed sites. Current methods for determining or monitoring toxic exposures to the warfighter in their working or living environment are not adequate to prevent serious health effects. Deployed personnel may be exposed to toxic chemicals as a result of industrial accidents, intentional or unintentional activities of enemy or friendly forces or sabotage. Rapid risk assessment of these scenarios requires the development of new testing methods. In order to prevent serious injury to the deployed warfighter exposed to toxic substances and to minimize mission degradation due to environmentally related adverse health effects, novel human monitoring methodologies that provide near real-time detection of potential toxic injury must be developed. It is necessary to devise methodologies that will predict or identify exposure of personnel to low concentrations of harmful substances before they cause harm to an individual. It is also important to identify methodologies that are relatively non-invasive, which could include collection of urine, blood, saliva or epithelial cells from humans. Emerging biotechnologies, such as toxicogenomics, proteomics and metabonomics will be investigated for their effectiveness to identify toxic effects upon the warfighter before they can induce a reduction in health and/or operational performance or before they can induce a disease process that would not manifest for several years.

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1.0 DEPLOYMENT TOXICOLOGY

Deployment of forces into unfamiliar, and potentially hostile, environments is inherently dangerous. An increasingly important issue in force protection is the toxicology associated with potential chemical and biological exposures at deployed sites. Making predictions on the probability and severity of adverse health effects from exposure to environmental contaminants at deployed sites is challenging because sufficient, accurate and reliable data are difficult, if not impossible, to obtain. The changing mission and increasing use of armed forces around the globe in non-battle operations has focused attention to threats of non-battle-related health problems (Rhomberg, 2000). Contaminated deployed environments, from theater-level combat to humanitarian missions, are becoming as hazardous as infectious disease risks in causing non-battlefield-related morbidity and mortality. Deployed personnel may be exposed to toxic chemicals as a result of industrial accidents, intentional or unintentional activities of various forces (enemy or friendly) or sabotage. Current methods of determining toxic exposures to the warfighter are not adequate to prevent serious health effects such as was induced by Agent Orange in Vietnam or to predict, prevent or rapidly rectify the scenario of the Gulf War Syndrome-like disease. The rapid risk assessment of these uncharacterized environments will require the development of new testing methodology proposed to be based on human health and mild perturbations of subclinical response. The emerging biotechnology techniques in transcription and/or protein expression (transcriptomics/genomics and proteomics), and metabonomics, involving nuclear magnetic resonance (NMR) and pattern recognition technologies, may be able to identify toxic effects occurring before they can cause any decrease in warfighter mission performance or induce a disease process that would not be manifested during duty, remain unnoticed until removal from duty, or after several years post-deployment. However, to accurately predict potential health effects to the warfighter using these emerging biotechnologies, accurate methods will be required to capture biologically relevant meaning from the generated data using these technologies. Bioinformatics, the use of computer science to obtain knowledge from biological data, is an integral part of successful methods development. Operationally, bioinformatics analysis is crucial in the collection of data and its manipulation to obtain understanding of biological processes. These technologies in combination will be used for the development of novel human monitoring methodologies that provide real or near real-time detection of potential toxic injury that will minimize mission degradation due to environmentally related adverse health effects by creating viable options for preventing or minimizing incapacitating exposures, or latent disease or disability in the months and years following deployment. A review of potential options and technologies for pre-deployment environmental surveillance, deployment exposure surveillance and post-deployment retrospective exposure surveillance has been published (Lippman, 2000).

2.0 WHAT IS UNDER OPERATIONAL TOXICOLOGY CONDITIONS?

One theory of toxicology can be perceived as being equally dependent on both time and dose. Although this concept has been around for over a hundred years, exceptions to this concept have been put forth, such as embedded in Haber's Rule (concentration (C) x duration (t) = a constant toxic effect (K)). Many toxicologists have focused on the dose-dependency of exposure, especially when effects are predominantly dose-, but not time-dependent. However, the present studies are focused on an effect at the lower portion of a response profile that would be reversible if given a modest amount of time. Most current risk predictions do not include time as a variable. While dose as a variable can be viewed as a simple function (# of molecules), time is much more complex with at least three time scales interacting with dose. The three time scales are: 1) toxicokinetic half-life (how the organism responds to a chemical), 2) toxicodynamic half-life (how is the organism affected by a chemical) and 3) frequency of exposure. According to Rozeman and Doull (1998), toxicity is defined as the accumulation of injury over short or long periods of time, which renders an organism incapable of

functioning within the limits of adaptation. This definition implies that both time and dose are functions of toxicity.

2.1 Dose-Time Relationship

The time-course for a toxicant in an organism (kinetics) is very different than the time-course of organism toxicity (dynamics). Underlying biological processes such as absorption, distribution, elimination, injury, recovery and adaptation all have different time scales. Consequences of interactions between toxicant and organism follow a causality route from toxicokinetics/toxicodynamics to manifestations of toxicity at the organism level. If recovery (adaptation, repair and reversibility) half-life exceeds the toxicokinetic half-life, then toxicodynamics becomes the rate-determining or rate-limiting factor. If the toxicokinetic half-life exceeds the recovery half-life, then toxicokinetics become the rate-determining/limiting factor. The present approach of early assessment using sensitive techniques to monitor organ-specific biomarkers and profile changes would be assessing warfighter pre-toxicology conditions in the dynamic mission environment. Because of this, baseline data and defining condition and outcome from invited survey data based on toxicodynamic organ responses and position of exposure will require secondary and tertiary follow-up sampling of the warfighter.

3.0 BIOTECHNOLOGY

It is not surprising that the word "biotechnology" connotes many definitions from the "omics" technologies to the cloning of animals. The definition of biotechnology that has been adopted by our laboratory is "the use of biological systems for the improvement of health and welfare of humans." A general perspective on biotechnology can be found in a recent publication by Frazier and Geiss (2000). Because the main focus of the present discussion pertains to novel biotechnology methods for potential monitoring of warfighter health, the derived biotechnology tools of genomics, proteomics and metabonomics will be briefly introduced.

3.1 Genomics

Major developments in large-scale genome sequencing and in the development of technology platforms to support it have had logarithmic growth over the past decade. A number of genomes have been successfully sequenced such as *Hemophilis influenzae* (Fleishman et al., 1995), yeast (Bussey et al., 1997), the nematode *Caterorhibditis elegans* (Anonymous, 1998) and recently, sequencing of the human genome. Significant portions and rough drafts of other important research organism genomes such as mouse, rat and zebra fish are presently known.

Differentially expressed genes in various types of samples (e.g. blood, target organ tissues, etc.) can be compared by conducting gene expression profiling studies. These studies provide cell/organ state information with respect to regulatory mechanisms and phenotypic activity of genes that comprise an organism's genome. Expression profiling techniques have become widely used in research and diagnostics to uncover disease pathways. DNA microarray analysis has become the most accepted technique among gene expression profiling techniques to initially monitor differential expression levels. DNA microarray analysis allows for profiling expression of tens of thousands of gene transcripts in parallel for high through-put screening, and is relatively easy to use.

Construction of gene microarrays for the quantitative assessment of transcriptional activity of tens of thousands of genes has resulted due to the availability of gene sequences and physical clones of the isolated

sequence from the coding region of these genes (Schena et al., 1995). A recent review of DNA microarray technology and its applications to mechanistic and predictive toxicology can be found in the literature (Pennie et al., 2000). We propose that the application of toxicogenomics will be highly beneficial in providing rapid profound assessment of toxicity in controlled laboratory exposure studies. Analogous to the area of drug discovery, increased mechanistic toxicity information will provide more accurate human risk assessments and direct research to biomarker pathways for identifying the best methods for monitoring human health.

3.2 Proteomics

Proteomics refer to the study of proteins expressed by an organism's genome within a certain expressed phenotype (e.g. liver cell , kidney cell, etc.). The determination of protein function is a major challenge of the post-genomic era. The speed at which target proteins can be isolated and identified will be the rate-limiting step in the establishment of proteomics as a useful diagnostic tool. The word "proteomics" was first reported in the literature by Waringer et al. (1995) and refers to the "total protein complement of a genome." Therefore, proteomics is the study of proteomes, and involves the measurement and analysis of proteins expressed by a cell at any given time. A review of proteome research can be found in the literature (Humphery-Smith et al., 1997).

While an organism's genome is relatively constant between cell types and over time, the proteome is dynamic and varies between cell types and within cells. Proteomes show a wide degree of variability in chemical characteristics such as size, solubility and concentration. A single cell contains approximately twenty thousand proteins and approximately one hundred to three hundred thousand proteins for an entire multicellular organism. These numbers are based on gene splicing prior to protein expression from the mRNA transcript, as well as from post-translational modification of the protein. Therefore, proteomics must not only concern itself with the formidable challenge of sequence diversity of proteins, but also the chemical diversity from post-translational modifications of proteins. These modifications include phosphorylation, glycosylation, acetylation, nitrosylation, proteolytic cleavage, co-factor insertion and many others, and along with up- and down-regulation of proteins are confounding factors for protein analysis.

Functional information for all cellular proteins will eventually be provided by proteomic studies. Currently, proteomic research is being driven by cell-mapping and protein expression strategies. With respect to the topic of medical surveillance technology, the protein expression strategy is the most viable. This strategy is concerned with monitoring global expression of large numbers of proteins within a cell or tissue, and quantitatively identifies pattern changes resulting from hazardous chemical or material exposures. The goal of expression profiling is to generate "protein fingerprints" that may provide insight into novel biomarkers of disease or toxicity.

3.3 Metabonomics

Metabonomics is an emerging biotechnology approach to enable high-throughput *in vivo* toxicology screening. This technology was pioneered by Jeremy Nicholson, Elaine Holmes and John Lindon at the Imperial College in London (Holmes and Shockor, 2000; Nicholson et al., 1999). A distinction must be made here before proceeding that is concerned with terminology. The quantitative measurement of the time-related multiparametric metabolic response of living organisms to pathophysiological stimuli or genetic modification is defined as metabonomics (Nicolson et al., 1999, Nicolson et al., 2002), while the assessment of metabolic composition of a cell is defined as metabolomics. Metabonomic studies investigate fixed cellular and biofluid concentrations of endogenous metabolites, as well as dynamic metabolite fluctuations, exogenous species, and molecules that arise from chemical rather than enzymatic processing (Lindon et al., 2003a). Metabonomics can be viewed as

a systems biology approach that can integrate divergent effects that occur over both time and space. Metabolomics, corresponding to the study of single cell metabolomes, can be thought of as a subset of metabonomics (Raamsdonk et al., 2001).

Metabonomics combines high resolution NMR with pattern recognition technology to rapidly evaluate the metabolic status of an animal. Using this approach, the onset, duration, severity and target organ localization can all be determined from a peripheral sample such as urine. Toxicants disrupt the normal composition and flux of endogenous biochemicals in, or through, crucial intermediary cellular metabolic pathways. These alterations may be reflected, either directly or indirectly, in the blood, which in turn may produce characteristic biomolecular traces in the urine. If a significant number of trace molecules can be monitored, the overall pattern produced may be more consistent and predictive than any single biomarker. Using high-field NMR, which is an essential component of metabonomics technology, obtaining comprehensive biochemical information is made possible. Proton (1H) NMR spectroscopy can detect all soluble proton-containing molecules with a molecular weight of 20,000 daltons or less at concentrations greater than 100 μM. The NMR spectra serve as the raw data for pattern recognition analysis, which reduces the complex multivariate data into two or three dimensions that can be readily evaluated for analysis comparisons. Acquiring this vast amount of information enables a potential for developing relatively high throughput, whole animal toxicity-screening throughout a toxic insult progression and resolution. The principles of this approach have been described (Anthony et al., 1994a; Beckwith-Hall et al., 1998; Holmes, et al., 1998a, 1992a, Holmes and Shockor, 2000; Nicholson et al., 1999). In addition, the use of NMR for metabolic analysis using biofluids has been recently reviewed (Reo, 2002; Lindon, et al., 1999). Extensive literature exists on the use of metabonomics procedures to evaluate nephrotoxicants (Robertson, et al., 2000; Holmes, et al., 1998b, 1992b; Anthony et al., 1994b) and hepatic toxicants (Robertson, et al., 2000; Beckwith-Hall et al., 1998; Holmes, et al., 1998a).

NMR analysis of biofluids (i.e. blood, urine, cerebral spinal fluid, etc) provides a window into the biochemical status of a living organism. The biofluid composition is modulated by the function of the cells responsible for its manufacture and secretion. This biochemical composition may be altered when organ damage occurs due to toxicity or disease. Such biochemical information can reflect the modes and severity of organ dysfunction. The use of NMR spectroscopy for the analyses of biofluids has recently been reviewed by Lindon, *et al*. (2003b).

NMR analysis requires minimal or no sample preparation, it is a nondestructive procedure and can usually be implemented in a noninvasive manner. Therefore, it is amenable for studies of biofluids, cell extracts, and for cell cultures and tissues *in vitro* or *in vivo*. The multinuclear capabilities of NMR provide various means to observe different chemicals. Most present published metabonomics work has exclusively used ^{1}H NMR for analyses of biofluids, but other nuclides (i.e., ^{13}C, ^{31}P, ^{15}N, ^{19}F, and ^{2}H) may provide additional information about various metabolite pools. The major limitations of the technique relate to spectral resolution and analyte sensitivity, both of which are improved by experimentation at high magnetic field strengths. Thus a majority of metabonomics studies have been conducted at ≥11.7 Tesla (a ^{1}H resonance frequency of 500 MHz or greater). Broadened NMR spectral lines can degrade resolution and the ability to differentiate metabolite signals. Factors influencing the NMR line-widths are due to molecular dynamics and include sample viscosity, macromolecules, binding of small molecules, compartmentaltization, and sample heterogeneity which is an inherit problem with cells and tissues.

4.0 BIOTECHNOLOGY SOLUTIONS FOR POTENTIAL MONITORING OF DEPLOYMENT HEALTH

Assessment of exposure is a critical element in risk assessment and management, and is especially important for armed forces deployed in hostile or uncharacterized sites. Enhanced capabilities that allow for real- or near real-time assessment of hazardous exposure will provide military field commanders with viable options for preventing or minimizing mission degradation due to these types of exposure, as well as preventing or minimizing latent disease or disabilities following deployment. The latter would have dire consequences on force readiness for future deployments, as well as affecting the quality-of-life of force members and their families. Therefore, monitoring hazardous exposures of deployed forces can be a valuable and cost-effective method for force protection.

Proceedings from a recent workshop entitled "Strategies to Protect the Health of Deployed U.S. Forces: Assessing Health Risks to Deployed Forces" (Rhomberg, 2000) suggests that during deployment data should be collected on personal exposures to on-site contaminants, using personal samplers and monitors, as well as the collection of exposure biomarkers whenever appropriate equipment, sampling opportunities, analytical methods and procedures are available. Furthermore, sampling strategy, because it will never be feasible to monitor all personnel, and documentation of force descriptors (e.g. age, ethnicity, genetic susceptibility, prior exposure and medical histories and other stress factors) cannot be overlooked.

Assessment of deployed armed forces for health effects resulting from exposure to unknown chemical and material hazards is challenging, particularly when the personnel being monitored serve as the biological monitors. One of the first challenges that is immediately apparent is the limited access to sources of biological material to be sampled. Biological samples collected from the warfighter must be obtained by non-invasive means when possible (e.g. urine, saliva, etc.), and only by minimally-invasive methods in all other cases (e.g. blood, spinal fluid, etc). Therefore, biological markers of exposure must be present in these types of biological materials if they are to be useful for monitoring human health effects due to hazardous exposures. Biomarker analysis will usually be performed on components of blood or urine due to sufficient quantities and relative ease of collection. Presented below are two examples from our laboratory using genomics and metabonomics technologies for potential toxicity assessments in rats exposed to a model liver toxicant. We are currently in the process of establishing proteomic capabilities for toxicity assessments.

4.1 Genomic Assessment of Toxicity Using Peripheral Blood Mononuclear Cells (PBMC)

The peripheral blood lymphocyte can be used for toxicogenomics assessment of hazardous exposures. Because these cells contain genetic material and circulate throughout the body, they can interact with toxic chemical (or metabolites there of) or biological agents. Lymphocytes have been shown to integrate exposure over extended time intervals because they are long-lived (Braselmann et al., 1994) and do not divide *in vivo*. A molecular-based approach such as genomics has the potential to be a highly sensitive technology for monitoring chemical exposure. Employing a highly parallel technique such as GeneChip analysis will enhance the probability of identifying biomarkers for chemical exposure.

A preliminary study was conducted in our laboratory to investigate whether PBMC could be used as a surrogate tissue for genomic monitoring of hazardous chemical exposures. A general overview of this genomic approach is shown in Figure 1. The objective of this study was to identify differential gene expression in PBMCs following exposure to a known liver toxicant (alpha-naphthylisothiocyanate; ANIT), and to identify variables useful for classification for pattern recognition techniques. PBMCs from Fisher 344

rats (3 rats/dose) were analyzed by transcriptomic profiling on an Affymetrix RAE230A GeneChip (~16,000 probe sets/chip) using an Affymetrix technology platform. Discriminant analysis and classification variable selection of the data were performed using various statistical algorithm software packages (Matlab, Insightful, Partek Pro, etc.).

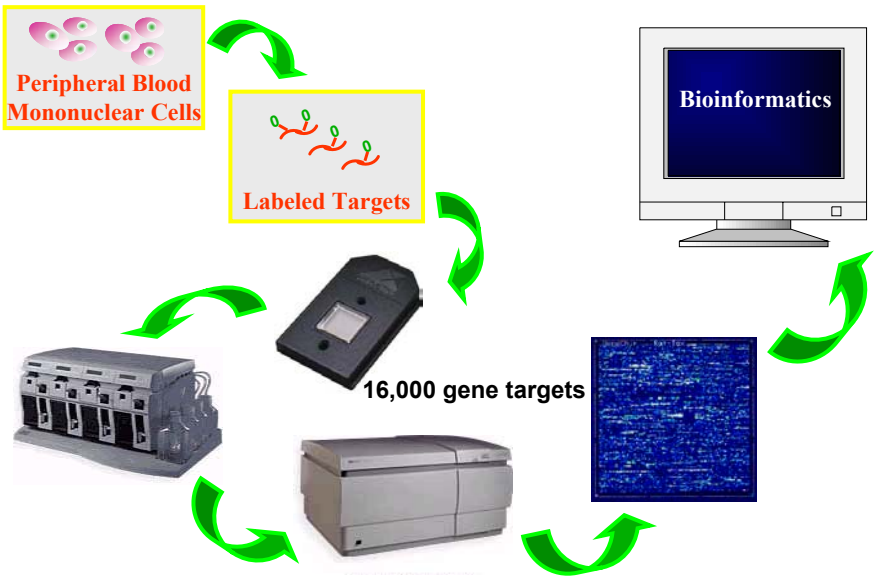

Figure 1: General overview of genomic assessment methodology for toxicity screening using peripheral blood mononuclear cells (PBMC) as a surrogate tissue

Changes in gene expression in rat PBMC following ANIT treatment (0.5, 1.0, 5.0 and 10.0 mg/kg) were analyzed using a t-test ($p < 0.05$), as well as the Affymetrix Comparison Analysis (Wilcoxon Signed Rank test, $p < 0.005$). To minimize potential false positives, a "double-double" approach was used to increase the stringency of the analysis (i.e. the differences in gene expression between the treated and untreated groups have to be significant in both the t-test and the Affymetrix Comparison Analysis in at least two doses). Thirty-two genes were identified that met this criterion, and these were then clustered into three groups using a correlation coefficient clustering technique based on their response to the ANIT treatment. Of these 32 genes, 14 showed significant changes at the lowest ANIT dose tested (Table 1). The clustering pattern of these genes is shown in Figure 2.

Examination of the 32 genes in these three clusters reveals that: 1) the genes in Cluster 1 are involved in biological processes specific for cell growth and maintenance including chromatin structure/dynamics, chromosome partitioning, transcription and translation, 2) the genes in Cluster 2 are involved in cell adhesion, immune response and protein synthesis/degradation and 3) the genes in Cluster 3 are involved in cell adhesion, chemotaxis, cytoskeleton, RNA splicing, protein synthesis/folding/degradation and metabolism of lipid and nucleotides. Interestingly, all nine genes in Cluster 1 showed significant changes at the lowest ANIT dose tested (0.5 mg/kg), while only 3 and 2 genes were significant changed in Cluster 2 and Cluster 3, respectively (see Table 1).

Table 1: Changes in Gene Expression in Rat PBMC Following 0.5 mg/kg ANIT Treatment

GENE CLUSTER 1

Catalase
Embigin, belongs to immunoglobulin super family; putative cell adhesion molecule
Pituitary tumor transforming gene; may play a role in cell growth and/or maintenance
Lamin A; nuclear envelope protein lamin, intermediate filament superfamily; involved in cell cycle/cell division, chromosome partitioning and nuclear structure
EST, moderately similar to human multisynthetase complex auxiliary component p18 (MCA3); a component of bifunctional and monospecific tRNA synthetase complex
EST, with strong similarity to human yippee protein; a novel family of putative zinc binding proteins highly conserved among eukaryotes
EST, with moderate similarity to human sirtuin 2, isoform 1; a novel receptor with seven transmembrane domains; may be involved in chromatin structure/dynamics and transcription
EST, unknown function/name
EST, unknown function/name

GENE CLUSTER 2

Allograft inflammatory factor-1 (AIF-1); expressed in inflammatory cells; may play a role in macrophage activation
Bst1: Bone marrow stromal cell antigen 1
Ly6 homolog RK3 precursor; superfamily: Ly-6 antigen; Ly-6 homology; GPI-anchored protein
Fc receptor, IgG, low affinity III; isoforms differ in IgG subclass-binding specificity; IgG Fc receptor of natural killer cells and macrophages
Ficolin 2 precursor (Collagen/fibrinogen domain-containing protein 2)
96KDa lysosomal membrane sialoglycoprotein precursor (lysosomal-associated membrane protein 2)
Cathepsin H, lysosomal proteinase; belongs to peptidase family C1.
60S Ribosomal Protein L21
EST, similar to goliath-related E3 ubiquitin ligase 4; may regulate growth factor withdrawal-induced apoptosis of myeloid precursor cells

GENE CLUSTER 3

Ferritin, heavy polypeptide 1; Ferritin subunit H
EST, moderately similar to mouse lymphocyte antigen LY-6E precursor; attached to the membrane by a GPI-anchor
Chemokine-like factor isoform a; chemoattractant for neutrophils, monocytes and lymphocytes; may play important roles in inflammation
Chemokine-like factor isoform a; chemoattractant for neutrophils, monocytes and lymphocytes; may play important roles in inflammation
Proteasome ring12 chain; superfamily: multicatalytic endopeptidase complex chain C5
SH-PTP2 protein tyrosine phosphatase, non-receptor type 11; Chaperonin subunit 4 (delta); molecular chaperone t-complex-type superfamily
Splicing factor, arginine/serine-rich 5; play a role in pre-mRNA splicing; may be required for cell growth/cell cycle progression
EST, similar to human splicing factor, arginine/serine-rich 4 isoform a
Beta-actin (cytoplasmic)
ESTs, highly similar to human AR16, actin-related protein 2/3 (ARP2/3) complex subunit 5 (16 kDa subunit)
Diphosphoinositol polyphosphate phosphohydolase type II
Nucleoside diphosphate kinase
Phosphoglycerate kinase; phosphoglycerate kinase superfamily
EST, unknown function/name

Significant at $p < 0.05$

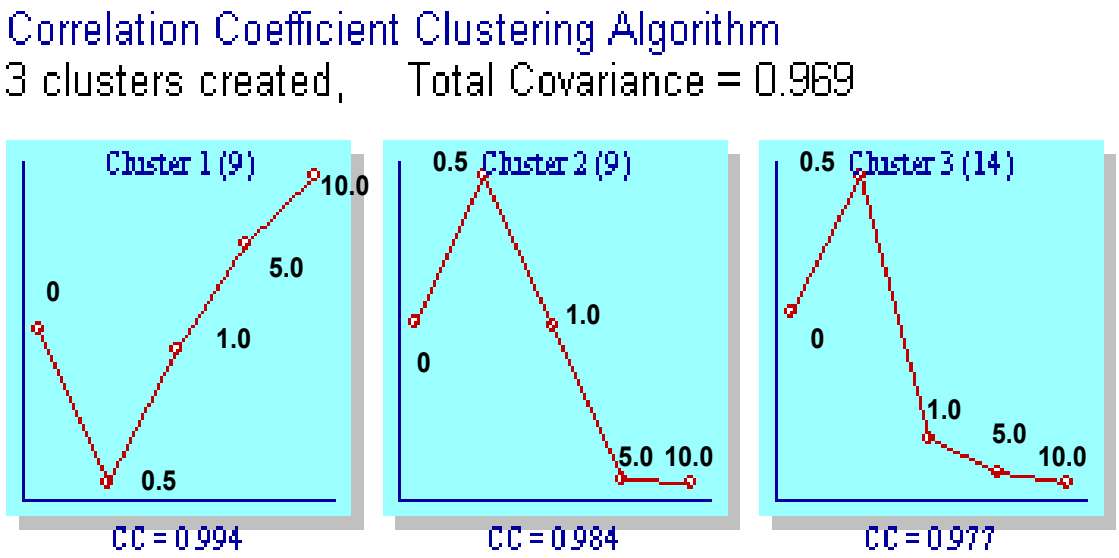

Figure 2: Clustering analysis of 32 genes with significant changes in expression. Graphs represent the dose response of these genes to ANIT treatment (0.5-10.0 mg/kg) when analyzed using correlation coefficient clustering technique. Three clusters, one up-regulated and two down-regulated, were observed.

4.2 Metabonomic Assessment of Toxicity Using Urine

Metabonomics is an approach used to characterize the metabolic profile of a specific tissue or biofluid. Because many biofluids can be easily obtained either non-invasively (urine) or minimally invasively (blood), they are typically used in metabonomic studies. However, other *in vivo* biofluids such as saliva, cerebrospinal fluid, bile, and seminal fluid, as well as *in vitro* biofluids such as cell culture supernatants and tissue extracts, can also be used. Metabonomics is an attractive approach to the study of time-related quantitative multivariate metabolic responses to pathophysiological processes because biological and chemical agents, or drugs, cause perturbations in the concentrations and fluxes of endogenous metabolites involved in critical cellular pathways. In other words, cells respond to toxic insult or other stressors by altering their intra and/or extracellular environment in an attempt to maintain a homeostatic intracellular environment. This metabolic alteration is expressed as a "fingerprint" of biochemical perturbations that is characteristic of the type and target of a toxic insult or disease process. These metabolic alterations are often seen in the urine as changes in metabolic profile in response to toxicity or disease as the body attempts to maintain homeostasis by eliminating substances from the body. Subtle responses to toxicity or disease under conditions of homeostasis also result in altered biofluid composition.

A study was conducted in our laboratory to investigate whether metabonomics could be used as an approach to monitor hazardous exposures to deployed forces. A general overview of our metabonomic experimental design is shown in Figure 3. The objective of this study was to develop NMR monitoring and pattern recognition technologies to screen urine/blood of deployed personnel to identify potential target organ toxicity resulting from low-level exposure to deployment-related chemicals. Using a Varian 600 mHz NMR

instrument, 24 h rat urines were collected daily over four days from animals exposed to a range of ANIT concentrations and analyzed. Pre- and post-exposure blood samples were also collected for clinical chemistry measurements, and tissue samples were processed for histopathological evaluations.

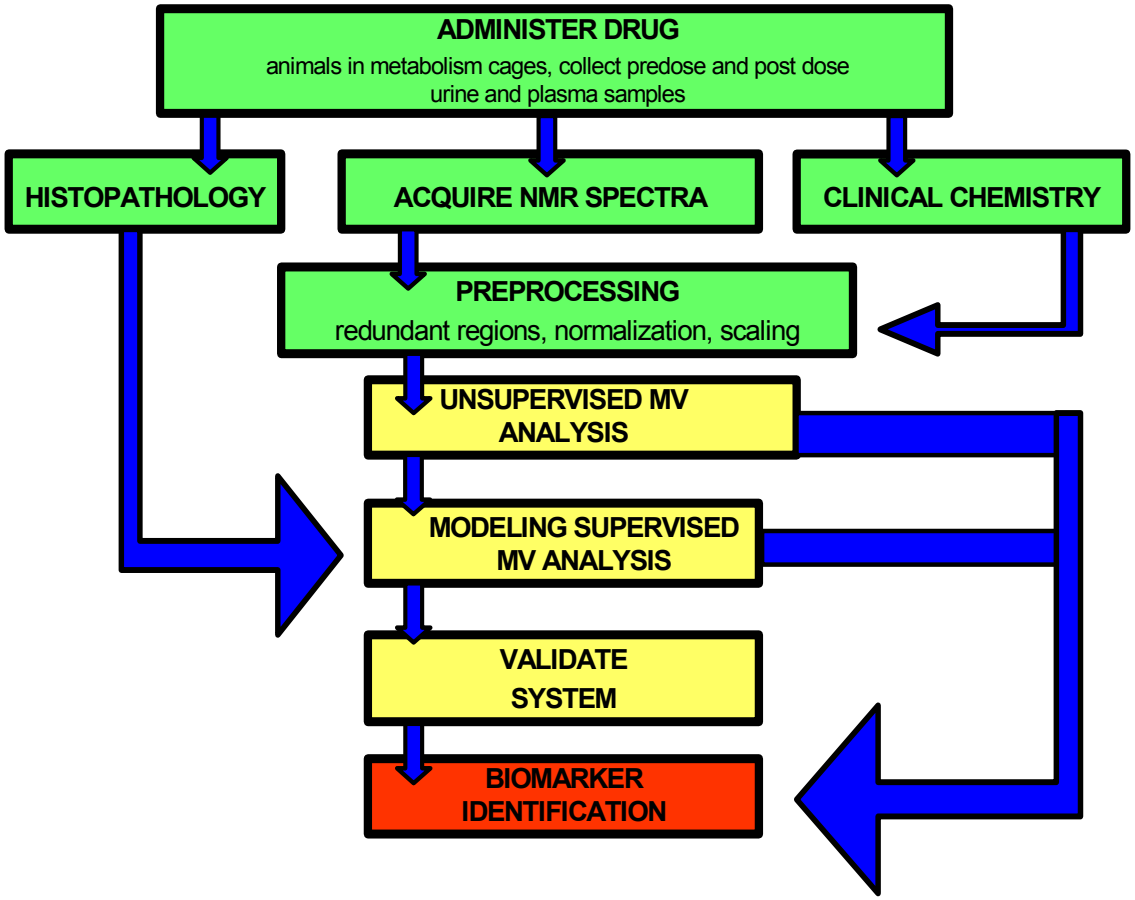

Figure 3: Overview of metabonomics study experimental design

Clinical chemistry analysis results from this study indicated that rats exposed to 100 mg/kg ANIT showed significant elevation of liver enzymes throughout the 4-day study period when compared to control (Table 2). Rats exposed to 50 mg/kg ANIT indicated significant elevation in liver enzymes following 24 h post-exposure that appeared to recover to normal levels by the end of the study at day 4. Rats exposed to ANIT concentrations less than 50 mg/kg did not demonstrate any significant change in liver enzymes throughout the study. The highest ANIT dose tested that did not induce any clinical effects on the liver was 20 mg/kg.

Table 2: Effect of α-Naphthylisothiocyanate (ANIT) Hepatotoxin on Selected Liver Clinical Chemistry Parameters (Mean ± SD)

	ALKP			ALT			AST	
Treatment	Day 1	Day 4		Day 1	Day 4		Day 1	Day 4
Control (Corn Oil)	280± 54	288± 25		38± 8	40± 11		82± 18	88± 18
ANIT (100 mg/kg)	370± 22	609± 155		152± 60	125± 37		278± 142	229± 101
ANIT (50 mg/kg)	380± 115	397± 50		232± 154	56± 16		552± 98	73± 13
ANIT (20 mg/kg)	259	335± 103		35	41± 19		91	72± 25
ANIT (10 mg/kg)	291± 20	332± 127		38± 6	36± 9		79± 10	88± 16
ANIT (1 mg/kg)	315± 22	293± 16		41± 11	39± 6		88± 23	68± 3
ANIT (0.5 mg/kg)	314	358± 129		43	29± 26		111	64± 16
ANIT (0.1 mg/kg)	-----	331± 163		-----	33± 6		-----	76± 9

(Mean ± SD) Significantly different than control ($p < 0.05$). ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase

At termination of the ANIT study (day-4), rats were euthanized and liver samples were fixed in 10% formalin. These samples were then processed for light microscopic evaluation. Liver tissues were also stained with hematoxylin and eosin prior to evaluation. Histopathology examination of the liver indicated that the severity score never exceeded "mild" in any of the animals exposed to ANIT. However, significant changes were only observed in rats exposed to ANIT at 50 mg/kg or higher (Table 3). The observed liver histopathology was consistent with effects known to occur in liver following exposure to the model choleostatic liver toxicant ANIT.

Rat urine was collected daily, beginning 24 h prior to exposure, over the course of the 4-day study from both control and ANIT treatment groups into cups containing 1.0 mL of 1% sodium azide maintained at 0°C. Rat urine collected at the end of each 24 h period was flash-frozen in liquid nitrogen and stored at minus 20ºC prior to analysis. Rat urine sample were analyzed using a Varian 600 mHz NMR instrument. Preliminary results of NMR analysis of urine collected from rats exposed to 100 mg/kg ANIT identified a number of

Table 3: Histopathology Evaluation of Rat Livers from Animals Exposed to Varying Concentrations of ANIT

Liver Dx	Corn Oil Only	0.1	0.5	1	10	20	50	100
mononuclear cell infiltrates, random	1 1 1	1	1		1			
biliary hyperplasia/hypertrophy		1	1			2 1	1 2 1	2 2 2 2 2 1 2 2 1
portal edema	1	1		1 1	1	2 1	1 2 1 1	1 1 1
portal inflammation	2 1	1	1 2		2	1 2	1 1 2 1	2 2 2 1 2 2 2
Animal Number	6 7 8 9 10 17 18 31 33 37	19 20 21	22 23 24	25 26 27	28 29 30	32 34 35 38	39 40 41 42	1 2 3 4 5 43 44 45 46

Significantly different from control ($p \leq 0.05$)

Note: Histopathology severity score (no value = no lesions, 1=minimal, 2=mild, 3=moderate, 4=marked, 5=severe). Dose above each non-control column is in mg/kg. Significance is based on a 2-tailed Wilcoxon Rank Sum test.

alterations in the NMR spectra, with effects on citrate predominating (Figure 4, Panel A). These alterations in urine profile were found to occur 24 h following expose and became more pronounced at 48 h post-exposure. In contrast, rat urine collected from animals exposed to 10 mg/kg ANIT; a dose that did not indicate any significant clinical effects over 4 days, indicated an altered NMR urine profile following 24 h post-exposure that was reversing back to normal at 48 h post-exposure (Figure 4, Panel B). Results to-date indicated that NMR analysis could identify altered urine profiles from rats exposed to the lowest ANIT concentration tested (1.0 mg/kg; data not shown).

5.0 CONCLUSION

Preliminary findings from our laboratory using genomics and metabonomic technologies indicated that both approaches appear to possess sensitive differential indicators of low-level chemical exposure. These approaches were able to detect changes in biofluid profiles (blood and urine) from animals exposed to the liver toxicant ANIT at doses well below that causing clinical effect. However, the sensitivities of these biotechnology approaches can be viewed as a double-edged sword. On the one hand, these technologies are very sensitive and give an early indication of exposure; while on the other hand, mission capability may be adversely impacted because field commanders constantly have to make decisions concerning personnel health status for assignments. Therefore, it is imperative that field commanders only are required to make this kind of decision when there are valid concerns of potentially hazardous exposures. Unquestionably, the detection of toxic insult by means of biochemical effects is difficult near the toxic threshold, yet these are frequently the most important effects to elucidate. One of the goals of our "omics" research is to identify significant alterations in biofluid profiles that occur near the threshold of toxicity for a particular target organ tissue that can serve as a biomarker of an exposure of concern. It is highly unlikely that a single "omic" technology will become a panacea for all future chemical toxicity testing studies. However, it is possible to use these biotechnologies in an integrated approach to explore the relationships between biofluid (or tissue) pattern profiles and toxicity that will lead to the generation of novel biomarkers of toxicity, and genetic identification of sensitive populations.

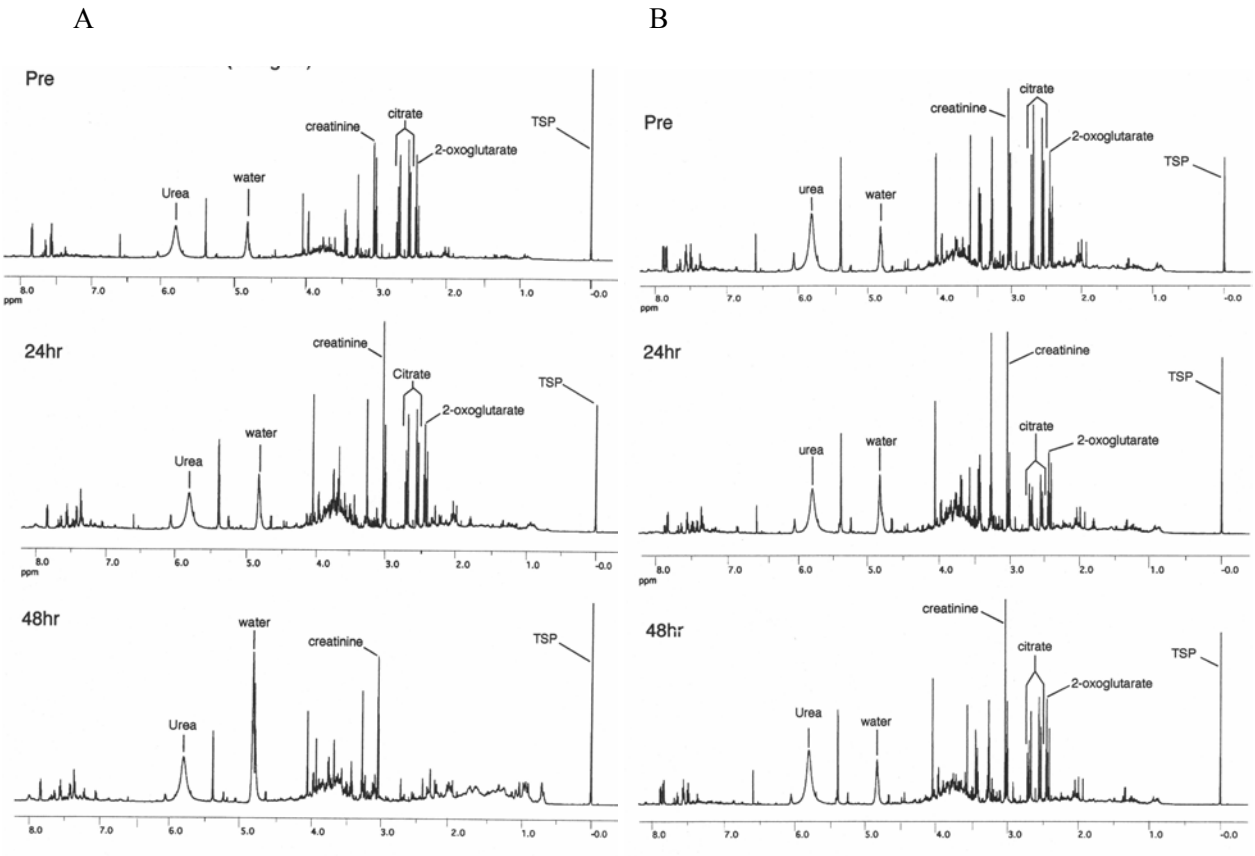

Figure 4: NMR spectra (600 mHz) of rat urine from rats exposed to ANIT at 24 h pre-exposure, and 24 h and 48 h post-exposure. A; 100 mg/kg ANIT. B; 10 mg/kg ANIT Note reduction in citrate peaks over time in the 100 mg/kg treatment group (Panel A) and the recovery of these peaks in the 10 mg/kg treatment group (Panel B) at 48 h-post exposure.

Applying any of the "omics" technologies will require generating databases for control animals and humans, disease states, and animals used in drug and toxicity testing. In addition to database generation, bioinformatic tools will need to be developed to statistically analyze the large volume of complex multivariate data generated by these technologies. Therefore, much work remains to be accomplished before these technologies can be used to provide biologically relevant predictions of hazardous risk due to chemical or material exposure. However, these technologies will eventually provide a global view of a complex organism's response to physiological stressors. This will lead to a better understanding of the relationship between gene function and metabolism in health and disease.

6.0 ACKNOWLEDGEMENTS

The opinions in this paper are solely those of the authors and do not reflect those of any US government agency.

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SYMPOSIA DISCUSSION - PAPER 19

Authors Name: Dr Del Raso (US)

Discussor's Name: LtCol Saracli (TU)

Question:
How does chronic exposure to toxic chemicals affect the diagnostic value of the system, especially for soldiers having possible chronic exposure history, previously?

Author's Reply:
It is envisioned that a metabonomics (e.g. NMR analysis of bio fluids) will be utilized to monitor military personnel for hazardous chemical exposure. This analysis would be performed pre -, during and post-deployment. Pre-screening will undoubtably require a questionnaire to determine pre-deployment status (ie.smoker, alcohol, diet, previous known exposures). If a person is deemed health but has a documented chronic exposure history, that person's urine profile pre-deployment would be reflected. If this base-line profile is significantly altered during post-deployment, it would be identified upon subsequent urine analysis. This assessment would be based on the assumption that all mechanisms-of-action per target organ have been characterized. This would result in the establishment of benchmark urine profile levels for each target-organ mechanism of toxicity that exceeds established benchmark. Therefore, unless chronic exposure results in the urine profile for a particular target organ mechanism of toxicity (that exceeds an established bench mark), it will not flag or be of concern.

Host Gene Expression Responses to Biothreat and Infectious Agents: Implications for Mathematical Modeling of *in vitro* Responses

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NATO CONFERENCE: OPERATIONAL ISSUES IN CHEMICAL AND BIOLOGICAL DEFENSE

Introduction: Detection of exposure to biological threat agents currently uses culture methods, immunoassay and gene amplification and these methods constantly are being perfected for greater sensitivity. However, recent events have demonstrated that assessing exposure to a biological threat agent well in advance of onset of illness or at various stages post-exposure would be an important capability to have among the diagnostic options. There is an urgent need for better diagnostic tools that will be sensitive, rapid and unambiguous to identify pathogen exposures and aid in triage of affected personnel. Rationale: Patterns of host cell transcription offers the potential for diagnosis of exposure/infection at a very early stage, even as early as ~1 hour post-exposure. The ability of diagnostic assays to identify infected individuals during the prodromal period is essential for successful treatment or intervention following exposure and infection with many disease-causing agents. The identification of early markers of infection offers great promise for revolutionizing disease diagnosis.

We have used gene microarray technology to characterized host gene expression responses (rather than direct pathogen exposure) to 14 biothreat and infectious agents using ex vivo exposures to each pathogen in peripheral blood mononuclear cells (PBMC). We found sets of genes that readily identify the pathogen used. By a thorough evaluation of healthy human baseline gene expression (using 75 diverse individuals), we identified genes with unambiguous responses for pathogen exposures. For 3 of these pathogenic agents, we have also carried out in vivo exposures in non-human primate models. Gene responses determined from blood drawn at various time periods post-exposure show clearly recognizable patterns of gene expression at remarkably early time frames. For example, B. anthracis exposure can be detected by direct pathogen identification at ~72 h post-exposure, while profiling of host gene expression responses identified an anthrax-specific pattern by at least 24 h. Due to the difficulties of such animal studies, we utilized bioinformatics approaches to correlate the in vitro to the in vivo gene responses and identified gene sets from in vitro studies that correctly predict pathogen exposure in vivo. Using the in vitro system at multiple concentrations and time periods post-exposure and mathematically modeling the gene responses to predict in vivo exposure patterns for those variables offers a means to establish a comprehensive approach for use of host gene expression responses for diagnostic purposes.

*Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition.

**Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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1. INTRODUCTION

The elucidation of the genetic events underlying the initiation and progression of human diseases is a critical step in designing better tools for diagnosis and treatment, and the advent of high-density microarray technology provides incredible opportunities for studying the disease process. High-throughput DNA and tissue microarray techniques promise to revolutionize the discovery and validation of novel molecular markers. Based on the information on the genome sequence as the outcome of the human genome project, functions of genes and proteins have been studied by expression profiling using microarrays, proteomics, single nucleotide polymorphism analysis, and coupled with bioinformatics. Along with advances in pharmacogenomics, these studies have raised the prospect of developing tests for individualized medicine based on genetic information, such as those predicting a person's susceptibility to diseases, or responsiveness to drugs for choice of treatment.

1.1 Global Molecular Analysis

Use of global methods that allow for high-throughput assessment of cellular function have become widely used by clinical and basic scientists working in both academia and the pharmaceutical industry. Differential display (DD)-PCR, DNA microarray, serial analysis of gene expression (SAGE), and subtractive hybridization are some of the techniques used to study global gene changes in numerous cell systems. The most efficient of these, microarray techniques, permits screening of ~40,000 genes simultaneously, producing information in an efficient manner. These are robust techniques, designed to highlight genes of interest that can be identified based on changes in expression profiles relative to a series of appropriate controls. Confidence levels are improved in studies that utilize sufficient numbers of replicates (4 experimental replicates, and having genes in duplicate on each slide, have been recommended for use in the microarray statistical package, Partek Pro, St. Louis, MO) and focus on clearly identified changes. For example, genes that have altered expression levels >5-fold, are reproducible in replicate samples. Similarly, genes having >3-fold changes are similarly altered in quite high percentages of the replicates. Focusing on the genes that have major changes in expression is a useful approach for selecting genes of interest for diagnostic purposes, but for therapeutic and mechanistic studies, important genes could be overlooked, since some minor change in gene expression level may result in a broad upstream change. Upon selection of genes of interest, complementary confirmatory techniques (RT-PCR, real-time-PCR, etc) are used to verify the response profiles. All of these techniques for global gene analysis are quite laborious, however, the resultant information obtained using these techniques is of incredible value for designing innovative diagnostic and therapeutic approaches. Studies utilizing each of these techniques have identified gene profiles that have been the basis for major advances in medical approaches

1.1.1 Current Uses Of Microarrays For Identification Of Unique Gene Patterns

Current publications show examples of the use of microarray technology for identification of differentially expressed genes in several sets of cancers (7-10). Cohen et al (11) have used this technology to identify genes induced by Listeria monocytogenesis in human promyelocytic THP1 cell line. Brutsche and colleagues (12) used peripheral blood mononuclear cells to study human B-cell isotype control and IgE production in patients with atopy and asthma and found significantly altered expression relative to healthy subjects. For several of these clinical studies, the number of patients enrolled are rather large (~100 for cancer studies) and ~20 for the asthma study; this number provides adequate statistical strength for correlation of gene patterns for differentiating the specific disease state

2. APPLICATION TO PROBLEMS OF IMPORTANCE FOR BIOLOGICAL DEFENSE

The threat of clandestine terrorist action using biological or infectious agents is especially a problem since many of these pathogens elicit non-specific flu-like symptoms early in infection; therefore, exposed individuals may not seek medical attention as a treatable stage. In contrast, an overt hoax or actual attack could result in people undergoing undue panic and medication during the long process of attempting to identify the pathogen.

Detection of exposure to biological threat agents currently uses culture methods, immunoassays and gene amplification, and these methods are constantly being perfected for greater sensitivity. However, recent events have demonstrated that assessing exposure to a biological threat agent well in advance of onset of illness, or even at various stages post-exposure would be an important capability to have among the diagnostic options. The ability of diagnostic assays to identify infected individuals during the prodromal period is essential for successful treatment or intervention following exposure or infection with many diseases-causing agents. The identification of early markers of infection offers great promise for revolutionizing disease diagnosis.

2.1. Goals of the Research Efforts in Applying Microarray Technology to Biodefense Problems

The ultimate goal of this research effort is to derive a series of gene expression responses to biological threat agents for use as diagnostic markers for exposure, as well as to analyze the information to design stage-specific therapeutic regimens. The rationale behind this approach encompasses several different scenarios.

2.1.1 Direct Pathogen Identification

It is frequently very difficult to identify an actual pathogen when a person presents with non-specific symptoms or if he/she “thinks” there might have been an exposure. Actual pathogen detection depends on its concentration in the matrix being examined, and that detection threshold may be reached too late for effective intervention in the case of many biological threat agents. Furthermore, when visiting a physician for even common illnesses, throats are swabbed and cultures are taken, and often a pathogenic agent is not identified, but rather a “footprint” that suggests a possible source of the infection is deduced. This presents difficulties when dealing with biological threat agents since many of these illnesses will not subside so readily as would be expected with common illnesses, such as influenza. Furthermore, many biothreat-induced illnesses start with flu-like symptoms, have varying latent periods and most proceed to cause lethality if countermeasures are not initiated early post-exposure (Figure 1).

Biological threat agents show different kinetic patterns of illnesses even though many start out with flu-like symptoms. In general, toxins are fast acting, while bacteria and viral exposures can vary in illness onset from days-months. Therefore, timing of exposure is very important; for each of these studies we characterized gene changes at early, mid, and late time points to understand the kinetics and pathogenesis of the illness. In our studies, we have observed some sets of genes that remain changed throughout the experiment, while other genes may show altered regulation at specific time frames post exposure.

2.1.2 Rapid Responses Are Essential For Biothreat Detection.

Early detection is very important to successfully treat exposure to biological threat and infectious agents. As was pointed out, actual pathogen detection is concentration-dependent and would most likely not reach sufficient levels in the early stages (13, 14). In our current work, in which we have exposed NHP to B. anthracis, the animals did not begin to how signs of even mild flu-like illness until 3-4 days. By 24 h, the

earliest time period examined in vivo, a robust gene expression response showed unique gene profiles (15). We are presently examining earlier time periods and believe that identification could confirm B. anthracis exposure even as early as 6-12 h, since the 24 h time period showed a robust and specific response

2.1.3 Unidentifiable Pathogens/ Bioengineered Agents.

One concern expressed by military experts relates to the fact that due to the biological sophistication of terrorist groups, chimera, mutants (natural or deliberate), or other modified pathogenic agents could escape detection if assayed with structural-based probes. We observed that certain sets of gene expression responses appear to be related to a "course of impending illness". For example, we have seen that "shock-inducing pathogenic agents" have common responses, and data mining shows a relationship of gene patterns that could indicate altered regulation of vascular tone, vascular leakage, pulmonary distress, etc. Our data suggest that gene patterns can reveal a profile related to the course of impending illness. If such a pattern is identified, medical personnel could initiate treatments to avert disastrous results.

2.2 Ongoing Studies Applying Microarray Technology To Biological Defense Problems.

We have selected agents from three different classes of pathogenic agents, bacterial pathogens, viral pathogens, and bacterial toxins and studied their host immune response in human PBMCs. 2.2.1 Bacterial toxins: The studied toxins can cause onset of acute illness within a few hours, but side effects can persist for many weeks. Staphylococcal enterotoxin (SE) B, a superantigen, Botulinum toxin A, a neurotoxin and Cholera toxin, an enterotoxin, each cause severe illness rapidly (Figure 1) and can proceed to death within a few days (13).

2.2.1 Biological Threat Agents Studied:

Bacterial. *Bacillus anthracis*: Inhalational Anthrax occurs once spores germinate in alveolar macrophages and are transported to the mediastinal lymph nodes. *Brucella melitensis*: Brucellosis is caused by gram-negative bacteria (B. melitensis), and is highly infectious via an aerosol route. *Yersinia pestis* causes pulmonary plague, which is fatal within a few days if left untreated (13). *Viruses*: Two RNA viruses have been studied for their induction of gene changes in human lymphoid cells. Venezuelan equine encephalitis (VEE) and Dengue infections each initially induce intense flu-like illness including headaches, fever and malaise. CFR could be a range from <1% (for dengue as well as for VEE) to in excess of 30% for dengue hemorrhagic fever. *Common illnesses*: Studies of febrile illnesses, influenza, adenoviruses and other illnesses are ongoing in various laboratories, including our own. Since so many biological threat agents begin with flu-like illnesses, it is extremely important to characterize these common illnesses so that genes can be selected that will unambiguously differentiate biothreat agents from these other febrile illnesses. Ongoing studies in other labs include gene profiling with samples from rheumatoid arthritis patients, general febrile illnesses and other illnesses that cause inflammatory responses.(16).

2.2.2 Bioinformatics and Data Mining:

As we started working on gene expression patterns we soon realized how important it was to have strong database and bioinformatics support. Additionally, we adapted or developed software necessary for analyzing, sorting, clustering and subjecting our data to statistical parameters. We have now accumulated increasingly more complex software from commercial sources for these analyses.

Our studies were carried out with 2-4 replicates of each of the 3-5 exposure times per pathogenic agent to provide extensive statistical strength. Furthermore, we have applied high-powered algorithms, designed to evaluate such massive data sets and focus on gene patterns that can identify gene patterns that will differentiate among the various pathogenic groups. In addition, our laboratory has developed automated

data mining tools in order to retrieve published information that could provide functional relationship links for the corresponding proteins (of genes significantly altered). We are collaborating with mathematicians who are independently scrutinizing the data and designing predictive modeling approaches.

2.3 Specific Findings In Our Laboratory:

Our objective was to create a library of host gene expression responses upon exposure to biological threat agents (15, 17). We first carried out these studies in vitro, exposing PBMC from healthy donors to the selected pathogen for various time periods, based on the experience of the pathogen specialist monitoring the course of the infection. We then selected 2 pathogenic agents for which NHP exposure closely replicates the illness as observed in humans. After aerosol exposure in NHP to these selected pathogenic agents, PBMC were collected at various time periods post-exposure and the resulting gene profiles determined. We find it necessary to use both in vitro and in vivo approaches for several reasons. First, there are some pathogenic agents for which an appropriate animal model does not exist. Secondly, extensive investigations of dose/time need to be carried out in vitro and selected information confirmed in vivo due to the costs and concerns associated with use of NHP. We expect that it may be very important to utilize species closely related to humans since certain differences in organ function are seen between rodents and humans. Indeed, we have rodent-specific gene arrays that are used for other studies, but we believe rodent models would not necessarily be appropriate for this objective. It is likely, though, that in vitro studies for such pathogenic agents may be the most reliable data that can be obtained and therefore, we are collaborating with mathematicians to develop sophisticated mathematical simulations with predictive modeling to provide important projections for these applications.

2.3.1 in vitro Studies:

For 8 pathogenic agents, we have compared gene patterns, sorting genes based on their function, and found that most pathogens upregulated genes coding for inflammatory mediators. This is not surprising and actually confirms what we know about the action of these biological threat and infectious pathogenic agents. Although inflammatory mediators may not differentiate among threat agents, they may best serve as markers indicating the progression of illness. There are many options for sorting and clustering the data utilizing specialized bioinformatics approaches; applying these techniques identified unique gene patterns induced by each agent. For some pathogenic agents, ~20 genes created a signature that was unique from the other 7 pathogens. Many of these genes relate to cell surface receptors and other molecules that the pathogenic agent utilizes to initiate its invasive action as well as to specific signaling sequences utilized by the pathogen.

2.3.2 in vivo Studies:

We selected 2 threat agents (B. anthracis and SEB) for which the NHP model closely reflects the illness induced in humans so that we could observe the type of gene patterns from in vitro studies that would likely be replicated in vivo (13, 18). Although the gene responses seen in vivo are very robust, encompassing both primary and secondary events, there are selected patterns that are remarkably replicated in vivo vs in vitro; these tend to relate to lymphoid receptors and the subsequent signaling cascades induced by each of these unique pathogenic agents. An example is a specific G-protein upregulated by SEB in NHP. It was not seen above background levels in our group of 75 healthy control individuals. For in vitro studies, the kinetics of this gene pattern is remarkably unique for SEB exposure. In this figure, the over-expression response was obvious in 30 min (3 h before onset of vomiting) and it persisted through at least 24 h (the last time period examined). For SEB, death from lethal shock is usually seen from ~42-60 h in NHP and information from accidental exposures of humans indicates a comparable time frame. Similarly, for B. anthracis exposure in NHP, unique sets of genes that show specificity for anthrax were identified even by 24 h post exposure, 2 days prior to identification of the

pathogen in body fluids and ~4-6 days prior to the onset of clinical signs of illness. Interestingly, we found genes that were early indicators of early exposure (24 h) vs later progression (72 h). This finding suggests that the "dose/time" combination may be able to be estimated based on gene patterns. These gene sets would not necessarily be the ones chosen as the markers of anthrax or other pathogen, but rather as staging indicators.

Biological threat agents show different kinetic patterns of illnesses even though many start out with flu-like symptoms. In general, toxins are fast acting, while bacteria and viral exposures can vary in illness onset from days-months. Therefore, timing of exposure is very important; for each of these studies we characterized gene changes at early, mid, and late time points to understand the kinetics and pathogenesis of the illness. In our studies, we have observed some sets of genes that remain changed throughout the experiment, while other genes may show altered regulation at specific time frames post exposure.

2.3.3 Therapeutic Implications.

As mentioned, several of these pathogenic agents eventually result in lethal shock and so we focused our attention on genes known to regulate vascular tone or contribute to vascular leakage. We then carried out analyses to determine what other genes co-regulated with known modifiers of the vasculature, focusing on genes whose expression level was altered preceding and following appearance of vascular lesions in the animal models. This led to some interesting findings relating to gene sets that may predict a "course of impending illness" and could provide the basis for designing stage-appropriate therapeutic intervention strategies. We are obtaining clinical samples from various "common" illnesses to further confirm that the gene patterns selected identify a specific biological threat or infectious agent. Another study in our lab involves determination of the effects of severe physical stress (to approximate battlefield conditions) on host gene expression responses to some of the same pathogenic agents that we have already studied.

2.3.4 Gene Profiles As A Baseline For The Health Human:

Our wealth of data obtained from PBMC gene profiles for 75 healthy human volunteers (diverse in ethnicity, sex and age) identified genes that are expressed at background levels in the healthy subjects but become massively overexpressed in response to one or more pathogenic agents (15, 17). These genes may provide unambiguous markers that could readily differentiate exposure to a pathogenic agent from unexposed individuals since the differences between "baseline vs exposure" is reproducible and easily obvious, despite ethnic/sex/age diversity. The functions of these genes, in general, relate to severe cellular responses to agonists/antagonists. We have confirmed altered regulation of selected genes using RT-PCR.

2.4 Gene Profiles Showing Unique Patterns For Specific Biothreat Agents

Figure 2 shows an example of a few selected genes that were expressed at near-background levels in the 75 healthy human volunteers, but show a unique pattern upon exposure to Brucella. As this graph illustrates, any 2 agents may show similarities in gene responses for some genes, the overall pattern is not the same when a sufficient number of genes are used for the indicators. A number of mathematical tools are used to select the specific genes and the number of genes needed to differentiate one pathogenic agent from the others. For example, using the Bonferroni analysis (19), 9 genes were identified as the minimal number to differentiate SEB from CT. Similarly, application of this approach to 4 of these pathogenic agents identified 25 genes as the minimum number to distinguish among those pathogenic agents.
Infectious Agents

As was pointed out, actual pathogen detection is concentration-dependent and would most likely not reach
2.1.3 Unidentifiable Pathogens/ Bioengineered Agents

One concern expressed by military experts relates to the fact that due to the biological sophistication of terrorist groups, chimera, mutants (natural or deliberate), or other modified pathogenic agents could escape detection if assayed with structural-based probes. We observed that certain sets of gene expression responses appear to be related to a "course of impending illness". For example, we have seen that "shock-inducing pathogenic agents" have common responses, and data mining shows a relationship of gene patterns that could indicate altered regulation of vascular tone, vascular leakage, pulmonary distress, etc. Our data suggest that gene patterns can reveal a profile related to the course of impending illness. If such a pattern is identified, medical personnel could initiate treatments to avert disastrous results.

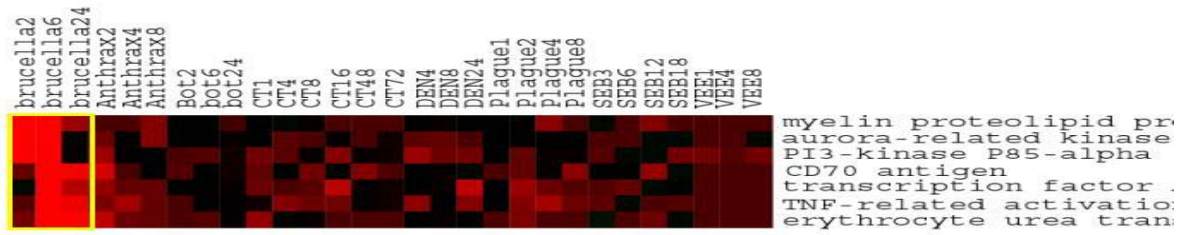

Figure 1. RT-PCR confirmation of selected gene responses initially identified from global gene analysis studies. In this graph, bright red indicates the highest upregulated genes. These genes were selected for this study since, for the 3 time periods of exposure to Brucella, they displayed massive up-regulation. Although there are some sporadic increases in these genes upon exposure to the other pathogenic agents, use of even these genes would enable one to discriminate brucella from the other pathogens. Our intention is to select ~50 such genes (which can be overlapping) to characterize each pathogenic agent

2.3.6 Studies From Other Laboratories

The Relman group has shown that there are distinct patterns of gene expression induced by Gram-negative and Gram-positive bacteria (16). They also compared heat killed isolates of Bordetella pertussis, E. coli, S. aureus and found gene patterns related to function to be altered in human PBMC. That laboratory has extensively focused on establishing profiles for "healthy" responses. DNA microarray has been used recently to report gene changes induced by various pathogens that give a unique gene response in dendritic cells (20). Other investigators are focusing on establishing gene profiles to characterize exposures to chemical warfare agents.

2.4 What are the Unique Problems Associated with Using Gene Arrays for this Purpose

What are the unique problems associated with using gene arrays for this purpose.Exposures to biothreat agents seldom occur in humans and therefore, such studies must rely on animal models that closely mimic the illness as it has been described to occur in humans. This usually involves use of non-human primates, and this is in contrast to many of the gene discovery studies previously described, in which the diseases

commonly occur throughout the general population. Furthermore, for some threat agents, representative animal models of the human illness are not available and so in vitro studies become the only alternative. Therefore, for host responses to biothreat agents, we first determined gene profiles upon exposure to peripheral blood mononuclear cells from healthy donors in vitro, and then compared those results with profiles obtained from in vivo exposure of non-human primates to the same biothreat agents

2.4.1 Kinetic Considerations

Gene patterns show time-related responses and Figure 1 illustrates the differences/similarities in onset of illness from the threat agents. During a terrorist attack, personal exposure dose would be unknown and time post-exposure could be unknown. One question we are attempting to answer relates to the findings in Figure 2, in which we demonstrate that if dose is constant, one can track time (post exposure) by selecting the secondary response gene profiles. We want to know if increased dose shows a similar change in gene profiles and if that change is reflected with essentially common gene sets to possibly indicate the "progression of illness."

2.4.2 Microarray Related Considerations

With the considerable amount of data generated by the different technologies using microarrays, it is obvious that the reading of the information and its interpretation and management through the use of bioinformatics is essential. Various techniques for data analysis are currently available and they require extensive evaluation before adapting to each project. Biochip and microarray technology have an essential role to play in the evolving trends in healthcare, which integrate diagnosis with prevention/treatment and emphasize personalized medicines.

2.4.3 Bioinformatics

Microarray data, by sheer virtue of their volume, can be better interpreted and analyzed using good statistical methods. Having replicates to validate and statistical analysis to define what is significant change is another critical issue in the field of microarray. Statistical analysis requires numerous replicates, which could sometimes be an issue with patient samples, monkey samples or experiments using rare pathogens. There are volumes that have been written regarding data processing, and we suggest those sources (21-29). Of course, some of the "noise" among samples involving patients relates to genetic variation, single nucleotide polymorphism (SNP) etc (30). Our studies have sought to diminish the noise from these factors by analysis of the unstimulated cells from 75 healthy volunteers (diverse in sex, age, ethnicity).

Another problem that needs some attention is how to translate these findings into a meaningful simple assay that can be performed by minimally trained personnel. In order to find a solution we decided to identify genes that are never expressed in normal healthy individuals but were massively overexpressed upon exposure to one or more pathogenic agent. The dramatic change in the expression levels aid in avoiding ambiguity.

We also believe that for the present time, the massive gene arrays are a preferred research laboratory tool, rather than a rapid clinical diagnostic. There are a many companies that aim to assess ~1000 genes simultaneously in nearly real-time and that approach offers good possibilities for early use of this technology in military efforts.

2.6. Conclusions and Future Plans.

In the past several years, our studies of host immune responses to biological threat agents have centered on signal pathways, cell mediators, and evaluation of gene responses upon exposure to biological threat

agents both in vitro and in vivo. Peripheral blood lymphoid cells can serve as a reservoir of historical information and are readily obtained from an exposed individual. We have shown that each pathogenic agent induces gene changes that are unique to that agent. These gene changes can be used for diagnostic markers as well as targeted for therapy.

By selecting genes that are expressed at low-levels in healthy volunteers yet show massively altered regulation upon exposure to one or more biothreat agents, we can establish gene patterns indicative of a particular agent, degree of exposure, etc and interpretation difficulties will be minimized. Similarly, we have also found a series of genes that are expressed at very high levels in these unstimulated samples, some of which become severely down-regulated upon exposure to one or more of the pathogens.

The studies thus far show specific gene profiles for the various biothreat agents that occur soon after exposure and these molecular changes in the PBMC appear far ahead of the actual symptoms or signs of illness, therefore, detection of a specific pattern early enough will permit these genes to be used as diagnostic and possibly therapeutic markers. We do intend to extend our studies to identifying distinct protein profiles induced by various biological and infectious agents, since they have shown interesting possibilities in detection of cancers (31). Use of such gene and protein screening techniques will give a new parameter to design new therapeutic approaches and design diagnostic tests for detection at any stage of the disease.

Microarray technology has important applications in pharmacogenomics: drug discovery and development, drug safety and molecular diagnostics. DNA chips will facilitate the integration of diagnosis and therapeutics, as well as the introduction of personalized medicines.

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SYMPOSIA DISCUSSION - PAPER 20

Authors Name: Dr Jett (US)

Discussor's Name: LtCol Saracci (TU)

Question:

1a) Do you believe that host response-based methods would replace non-cultured, agent-based amplification methods, such as PCR?

1b) Especially, respect to the high sensitivity of these amplification methods.

2) How can you make discriminations among micro-organisms which use same/similar pathogenic mechanisms in host, probably causing similar host responses?

Author's reply:

1a) All methods will have an important miche. I do not expect host-responses to replace, but rather supplement other methods.

1b) Even at high sensitivity, the best PCR amplifications must have the pathogen to result in a signal. It takes time before even any pathogen can be detected in (small amounts of body fluids)

2) We can discriminate among very closely related pathogens (such as staphenrerotoxim A & B). This discrimination may relate to receptors G – proteins and signals although the eventual outcome is vascular leakage, leading to DIC, multi-organ failure and death. We have seen vast differences in gene responses to LPS, SEA, SEB especially relating to CD markers, G Proteins, etc. Inflammatory medicators do not easily discriminate and we would not expect them to do so. However, they show the severity of the exposure and "stage" of impending illness. Although therapy targeting antictokine effects has been shown in the literature (Karima – 1996) to not have a significant effect on outcome. Other therapeutic targets (microemboli using anti thrombin or the Eli Lily drug, Xigris) may be common, but the timing is different for each shock – inducing pathogen.

Authors Name: Dr Jett (US)

Discussor's Name: Capt (USN) Campbell (US)

Question:

Dr. Ken Olden, NIEHS (US) started an initiative called "The Environmental Genome Project" to identify Human Genes that conferred resistance of susceptibility to environmental threat. Are you working with NIEHS to measure expression of any "Environmental Genes" that their project identifies?

Author's Reply:

I have lost contact with NIEHS, and that is regrettable.

I am so pleased that you brought up this point and I will pick up that thread again. The funding from the NIEHS Project will be important, especially when evaluating "Project Normal" among humans.

Field Evaluation of a Deployable RT-PCR Assay System for Real-Time Identification of Dengue Virus

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ABSTRACT

*Dengue fever and the more severe form of the disease, dengue hemorrhagic fever, occurs in tropical and subtropical regions globally through infection by one or more of four viral serotypes, DEN-1, DEN-2, DEN-3 and DEN-4. Viral transmission to humans is through mosquito vectors, primarily **Aedes aegypti**. Dengue universal and DEN 1-4 serotype specific fluorogenic, real-time RT-PCR assays and positive control nucleic acid were field-formatted by lyophilization and adapted for use on a field-durable, real-time, fluorimetric thermocycler. Nucleic acid extract isolated from DEN1-4 inoculated **Aedes aegypti**, field-captured **Aedes aegypti** and **Culex** spp, and field-captured **Aedes aegypti** and **Culex** spp spiked with total nucleic acid from DEN1-4 inoculated **Aedes aegypti** were used to construct a test panel (n = 64). In blind testing each of the five assays were 100% concordant in **in vitro** sensitivity and 100% concordant in specificity with dengue virus inoculated and spiked field-captured mosquitoes. A field-captured **Aedes aegypti** pool consisting of five females and two males was identified as dengue virus positive by the dengue universal assay; however, serotype identification was inconclusive. This study demonstrates the operational utility of a deployable assay system for rapid, sensitive, and specific screening and serotype identification of dengue virus in mosquito vectors.*

Introduction

Dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) now represent the most significant mosquito-borne viral disease threatening two-fifths of the world's human population (1, 2, 3). Infection with dengue virus causes various degrees of illness ranging from mild febrile discomfort through potentially fatal hemorrhagic disease (1). Vaccines are in development that shows promise (4, 5) however the current method of prevention and treatment is vector avoidance and patient management, respectively (6, 7). Antibody cross-reaction occurs across the *Flaviviridae* family creating ambiguity in immunoassay-based dengue virus surveillance and DF/DHF diagnosis (8, 9). To aid in clinical diagnosis, sensitive and specific dengue virus reverse transcriptase-polymerase chain reaction (RT-PCR) assays have been developed on laboratory-based instrumentation (10, 11, 12, 13, 14, 15, 16).

In areas where laboratory-based diagnostics are not available, deployable surveillance capability is an essential element in achieving timely assessments of transmission risk and time-critical implementation of appropriate

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mosquito control measures and clinical response in a potential outbreak situation (17, 18, 19). Development of pathogen identification assays on field-durable instrumentation fulfils a fundamental requirement in the realization of a complete field-deployable assay platform. While field-durable instrumentation provides deployable assay capability, an omnipotent field-deployable assay platform requires additional components including thermal-stable assay reagents and control nucleic acid, and field-formatted nucleic acid isolation technologies. Surveillance studies conducted in austere environments present logistical and operational constraints that make it impractical to transport, store, and prepare PCR reagents and control nucleic acid. Where resources for cold chain maintenance are unreliable or nonexistent, unknown variables in enzymatic activity and control nucleic acid integrity result. Moreover, sample integrity comes into question where sample collections are made distant from the laboratory or support facilities. Exposure to ambient temperature and freeze-thaw occurrences during transportation often presents an unknown variable in nucleic acid integrity of the sample.

This paper describes a deployable assay platform for rapid, sensitive, and specific screening and serotype identification of dengue virus in mosquito vectors.

Materials and Methods

Primer and probe design

Universal dengue virus assay primer and probe sequences were designed *de novo* by aligning homologous genomic regions of serotypes 1-4 that excluded other clinically significant flaviviruses. Alignments were compared visually using the Clustal algorithm (20) in the MegAlign program of DNA Star software (Perkin Elmer, Norwalk, Conn.)(21). Maximally conserved oligonucleotide sequences were chosen from dengue virus type 1-4 genomes downloaded from Genebank accession numbers U88536, M19197, M93130, AF326825, respectively. Yellow fever, JE, WN, and SLE virus type strain genomic sequences were aligned and visually evaluated to validate heterology with universal primer and probe sequences, Genebank accession numbers X03700/K02749, M18370, M12294/M10103, AF242895 respectively. Universal primer and probe sequences can be requested through the corresponding author. Serotype specific primer and probe sequences were obtained from the literature, [DEN-1 (10); DV2 (11); DEN-3 (10); DEN-4 (10)], and heterology validated as described above. All probes reported here are dual fluorogenic label designed with a 5' reporter dye, 6-carboxyfluorescein (FAM), and 3' quencher dye, 6-carboxytetramethylrhodamine (TAMRA) [22].

Universal and serotype specific primers and probes sequence heterology with genomic sequences of closely related species through diverse genera were validated by BLAST database search (BLAST, Madison, Wisconsin) [23]. Melting temperatures were quantified and the absence of significant primer dimerizations and secondary structure (hairpin) formations were confirmed with PrimerExpress software (PE Applied Biosystems, Foster City, Calif.). Primers and probes were synthesized and quality control conducted commercially (Synthetic Genetics, San Diego, Calif.). The efficacy of assay designs were validated in laboratory-based testing with multiple strains of dengue serotypes 1-4, yellow fever, Japanese encephalitis, West Nile, and St. Louis encephalitis viruses as well as dengue virus infected clinical specimens, vector species and human genomic DNA (McAvin JC, Escamilla EM, Blow JA, et al *Rapid Identification of Dengue Virus by RT-PCR Using Field-Deployable Instrumentation* (Submitted to Military Medicine 2004 Jan.).

Dengue Virus Inoculated Mosquitoes and Assay Test Panel

To field-site validate dengue virus field-formatted RT-PCR assay *in vitro* sensitivity and specificity Den1-4 inoculated *Aedes aegypti* were obtained from the Mosquito Biology Section, Department of Entomology, US

Medical Component Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. One- to 6–day-old adult female *Aedes aegypti* were inoculated intrathoraciclly (24) with one of the following viruses: DEN-1, DEN-2, DEN-3, DEN-4. Mosquitoes were held in cardboard cages, provided a carbohydrate source (either apple slices or a gauze pad soaked in a 10% sucrose solution) and a water-soaked cotton pledget, and held at 26°C for 7 days. Mosquitoes were then killed by exposure to -20°C for 5-10 minutes and 1-6 legs were removed for analyses. Legs were triturated in grinding diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts, $NaHCO_3$, and antibiotics) and tested for the presence of virus by plaque assay on LLC-MK-2 cells. One virus-inoculated or an unexposed mosquito body was added to each pool of non-infected mosquitoes. These were placed in sterile 1.5-ml Eppendorf tubes and triturated in 750 µl TrRIzol-LS (Life Technologies, USA).

To evaluate the efficacy of the field-deployable assay system a blind test panel of 64 nucleic acid extracts was prepared from DEN 1-4 inoculated *Aedes aegypti*, field-caught *Aedes aegypti* and *Culex* spp, and field-caught *Aedes aegypti* and *Culex* spp spiked with DEN 1-4 inoculated *Aedes aegypti* total nucleic acid (Table 1). The study site was Kamphaeng District, Thailand, August 2003. Mosquitoes were collected primarily in villages inhabited by patients with classic dengue fever symptoms. Mosquitoes were captured by battery powered hand-held aspiratorswithin village homes, mosquitoes pooled by residence, and transported live to the deployed assay platform.

RNA preparation

Total nucleic acid extracts were prepared with a commercially available, thermo-stable, preformatted viral RNA purification kit, QIAamp viral RNA mini kit (QIAGEN, Valencia, California). Mosquitoes were homogenized in 560 µl AVL Buffer/Carrier RNA component of the kit with sterile, RNase free pestles and 1.5 ml tubes. Homogenate was cleared by centrifugation on a table-top centrifuge at 12,000 rpm for 60 seconds and total nucleic acid extract prepared following the manufacture's spin protocol. Each extract was suspended in 60 µl of elution buffer.

Reaction conditions

Each sample was labelled under code and subjected to blind testing. Assays were conducted on the Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) [Idaho Technology Incorporated, Salt Lake City, Utah; www.idahotech.com] with lyophilized proprietary master mix components (Idaho Technology Incorporated, Salt Lake City, Utah) (25). Master mix reaction solution was prepared by adding 40 µl of PCR grade water to lyophilized master mix reagent and dispensing18 µl volumes into optical capillary tubes. To each capillary, 2 µl of RNA extract added from specimens or 2 µl PCR grade water for no template controls (NTC). Capillaries were placed in tabletop centrifuge and spun for 2-3 seconds at 3000 rpm to drive the reaction mixture to the bottom of the capillary. The dengue virus universal assay lyophilized master mix included thermo-stable, hydrolytic enzyme shielded, dengue virus Armored RNA® control template (Ambion RNA Diagnostics, Austin, Texas) (26) and was prepared by adding 40 µl of PCR grade water.

A standardized RT-PCR thermal cycling protocol was established that consisted of RT at 60°C for 20 minutes followed by an initial cDNA denaturation at 94°C for 2 minutes, and PCR for 45 cycles at 94°C for 0 seconds of template denaturation and 60°C for 20 seconds of combined annealing and primer extension. A single data point at the end of each annealing-extension cycle was collected and reported as TaqMan probe fluorescence released by 5'-nuclease activity during primer extension. Fluorimeter gains were set at 8-2-2 on channels 1, 2, 3 respectively. Protocols for all five assays are identical with the exception of DEN-3, extension temperature

was 66°C. The criterion for a positive result was a significant increase in fluorescence over background levels as defined by an algorithm provided in the R.A.P.I.D analytical software (Roche Molecular Biochemicals, Indianapolis, Ind.).

Results

In preliminary field-site testing with DEN 1-4 inoculated *Aedes aegypti* control samples, all five field-formatted dengue virus assays had an *in vitro* sensitivity and specificity of 100%. Total nucleic acid extract isolated from DEN1-4 inoculated *Aedes aegypti*, field-captured *Aedes aegypti* and *Culex* spp, and field-captured *Aedes aegypti* and *Culex* spp spiked with extract from DEN 1-4 inoculated *Aedes aegypti* were used to construct a blind test panel (Table 1). In blind testing, the dengue universal assay demonstrated an *in vitro* sensitivity of 100% (14/14) and 100% specificity of (64/64) with DEN1-4 inoculated *Aedes aegypti* and field-captured *Aedes aegypti* and *Culex* spp spiked with DEN 1-4 inoculated *Aedes aegypti*. Field-formatted DEN-1, DV-2, and DEN-4 serotype specific assays demonstrated an *in vitro* sensitivity of 100% (3/3) and specificity of 100% (64/64) with DEN1-4 inoculated *Aedes aegypti* and field-captured *Aedes aegypti* and *Culex* spp spiked with DEN 1-4 inoculated *Aedes aegypti*. The field-formatted DEN-3 assay demonstrated an *in vitro* sensitivity of 100% (4/4) and specificity of 100% (64/64) with DEN1-4 inoculated *Aedes aegypti* and field-captured *Aedes aegypti* and *Culex* spp spiked with DEN 1-4 inoculated *Aedes aegypti*. A field-captured mosquito pool (#47) was identified as dengue virus positive by the dengue virus universal assay. Serotype identification was inconclusive. Sample processing and RT-PCR required less than two hours.

Discussion

The study site was Kamphaeng District, Thailand, August 2003. Ambient temperature and humidity conditions were typical of summer weather in tropical regions, 28-38 degrees centigrade and 80-100%, respectively. Logistical and operational constraints inherent to a developing region made it impractical to transport, store, and prepare conventional PCR reagents and control nucleic acid because cold chain resources were unreliable.

The R.A.P.I.D. provided field-durable, real-time, fluorimetric PCR/RT-PCR instrumentation and commercially available, “off the shelf”, thermo-stable nucleic acid isolation technology facilitated field-collected sample processing. We integrated lyophilized dengue virus RT-PCR assays and thermo-stabilized positive control to construct a fully deployable assay system. The validity of lyophilized dengue virus assays was field demonstrated with thermo-stable, hydrolytic enzyme shielded, Armored RNA® control template (Ambion RNA Diagnostics, Austin, Texas) lyophilized with master mix reagents (Idaho Technology Incorporated, Salt Lake City, Utah). These provided master mix reagents and positive control that were easily transported, field-sustainable, and only required hydration and addition of sample template prior to the RT-PCR. Evaluation of nucleic acid isolation reagents showed promise for field-collected sample processing.

In this field evaluation, a field-captured mosquito pool consisting of five female *Aedes aegypti*, including one blood fed, and two male *Aedes aegypti* was identified as dengue virus positive by the dengue virus universal assay however serotype identification was inconclusive. That the dengue universal and all four serotype specific assays demonstrated 100% concordance in *in vitro* sensitivity and 100% concordance in specificity with DEN1-4 inoculated *Aedes aegypti* and field-captured *Aedes aegypti* and *Culex* spp spiked with DEN 1-4 inoculated *Aedes aegypti* suggests a false negative in serotype identification and the source of error is experimental, most probably due to a sample number recording error. Additional field-testing is planned.

This study demonstrated the operational utility of a deployable assay platform for the surveillance of dengue virus, potential for applications in surveillance of other significant vector-borne pathogens, and promise as an aid in traditional clinical laboratory diagnostics.

References to trade name, vendor, proprietary product or specific equipment is not an endorsement, a guarantee or a warranty by the U.S. Department of the Defense, Army or Air Force, and does not imply an approval to the exclusion of other products or vendors that also may be suitable. The conclusions and opinions expressed in this document are those of the authors. They do not reflect the official position of the U.S. Government, Department of Defense, Joint Program Office for Chemical and Biological Defense, U.S. Air Force, U.S. Army, or the Association of Military Surgeons of the United States.

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Thanks to Lt Col James W. Jones, Ph.D., Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand for providing the study site and dengue virus inoculated *Aedes aegypti*.

Table 1 RT-PCR Results

#	Genus	*species*	Sex	Collection location	DU-JCM	DEN-1	DV-2	DEN-3	DEN-4
1	*Aedes*	*aegypti*		DEN-1 Inoculated	+	+			
2	*Aedes*	*aegypti*		DEN-2 Inoculated	+		+		
3	*Aedes*	*aegypti*		DEN-3 Inoculated	+			+	
4	*Aedes*	*aegypti*		DEN-4 Inoculated	+				+
5	*Culex*	*quinquefasciatus/tritaeniorhynchus*	3 F	Village 1/House 053 (1/053)					
6	*Aedes*	*aegypti*	1 F	1/059					
7	*Culex*	*tritaeniorhynchus*	1 F	1/059					
8	*Culex*	*tritaeniorhynchus*	5 F	1/058					
9	*Culex*	*tritaeniorhynchus* spiked with DEN-1	4 M	1/058	+	+			
10	*Aedes*	*aegypti*	1 F	1/058					
11	*Aedes*	*aegypti* spiked with DEN-3	1 M	1/058	+			+	
12	*Aedes*	*aegypti* spiked with DEN-4	2 M	1/050	+				+
13	*Aedes*	*aegypti*	6 F	1/049					
14	*Aedes*	*aegypti*	1 M	1/049					
15	*Culex*	*tritaeniorhynchus*	1 F	1/049					
16	*Culex*	*gelidus*	2 F	1/054					
17	*Culex*	*tritaeniorhynchus*	8 F	1/054					
18	*Aedes*	*aegypti*	1 F	1/054					

#	Genus	*species*	Sex	Collection location	DU-JCM	DEN-1	DV-2	DEN-3	DEN-4
19	*Aedes*	*aegypti* spiked with DEN-2	5 M	1/054	+		+		
20	*Aedes*	*aegypti*	7 F	1/056					
21	*Aedes*	*aegypti*	1 M	1/056					
22	*Culex*	*tritaeniorhynchus*	1 F	1/056					
23	*Culex*	*tritaeniorhynchus*	10 F	1/057					
24	*Culex*	*tritaeniorhynchus*	10 F	1/057					
25	*Culex*	*tritaeniorhynchus*	12 F	1/057					
26	*Aedes*	*aegypti*	5 F	1/057					
28	*Aedes*	*aegypti*	2 F	1/055					
29	*Aedes*	*aegypti*	9 F	1/067					
30	*Aedes*	*albopictus*	1 F	1/067					
31	*Culex*	*tritaeniorhynchus/geldidus*	9 F/ 2F	1/060					
32	*Culex*	*tritaeniorhynchus/vishnui*	2 F/2M	1/150					
33	*Aedes*	*aegypti*	3 F	1/150					
34	*Aedes*	*aegypti*	1 F	1/051					
35	*Culex*	*quinquefasciatus/tritaeniorhynchus*	2F/2F	1/062					
36	*Aedes*	*aegypti*	2 F	1/066					
37	*Aedes*	*aegypti*	1 F	1/066					
38	*Culex*	*quinquefasciatus/tritaeniorhynchus*	2F/3F	Village 8 House 50 (8/50)					
39	*Culex*	*quinquefasciatus*	10 F	Village 8 House 50 (8/50)					
40	*Aedes*	*aegypti*	2 F	8/50					
41	*Aedes*	*aegypti*	10 M	8/50					
42	*Aedes*	*aegypti*	2 F	8/252/11					
43	*Aedes*	*aegypti*	2 F (PBM)	8/252/12					
44	*Aedes*	*aegypti*	3F/3M	8/32					
45	*Aedes*	*aegypti*	1F/2M	8/45					

#	Genus	*species*	Sex	Collection location	DU-JCM	DEN-1	DV-2	DEN-3	DEN-4
46	*Aedes*	*aegypti*	2F/2M	8/19					
47	*Aedes*	*aegypti*	5F(1FBM)/2M	8/25	+				
48	*Aedes*	*aegypti*	2 F	8/51					
49	*Aedes*	*aegypti*	1F (BF)/6M	8/22					
50	*Aedes*	*aegypti*	1 M	8/21					
51	*Aedes*	*aegypti*	1 F	AFRMIS- Inoculated Dengue 3	+			+	
52	*Aedes*	*aegypti*	1 F	AFRMIS- Inoculated Dengue 2	+		+		
53	*Aedes*	*aegypti*	1 F	AFRMIS- Inoculated Dengue 1	+	+			
56	*Aedes*	*aegypti*	1 F	Village 642 House 229 (642/229)					
57	*Culex*	*quinquefasciatus*	4 F	642/231					
58	*Culex*	*quinquefasciatus*	12 M	642/231					
59	*Culex*	*quinquefasciatus*	1 F (BF)	642/231					
60	*Culex*	*quinquefasciatus*	1 F (PBM)	642/231					
61	*Culex*	*quinquefasciatus*	1 F (PBM)	642/231					
62	*Culex*	*quinquefasciatus*	1 F (BF)	642/231					
63	*Culex*	*quinquefasciatus*	1 F (PBM)	642/231					
64	*Culex*	*quinquefasciatus*	1 F	642/231					

F=Female

M= Male

BM= Bloodmeal

PBM = Partial Bloodmeal

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Identification of *Aedes aegypti* and its Respective Life Stages by Real-Time PCR

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ABSTRACT

*An **Aedes aegypti** specific fluorogenic probe hydrolysis (TaqMan) PCR assay was developed for real-time screening using a field-deployable thermocycler. Laboratory-based testing of **Ae. aegypti, Ae. aegypti** (Trinidad strain); **Culex pipiens; Culex pipiens quinquefasciatus; Anopheles stephensi; Ochlerotatus taeniorhynchus** individual adult mosquitoes and mixed pools (n=10) demonstrated 100% concordance in both **in vitro** sensitivity (6/6) and specificity (10/10). A single adult **Aedes aegypti** was identified in a pool of 100 non-**Aedes aegypti** mosquitoes. The limit of detection of **Aedes aegypti** egg pools was 5 individual eggs. Field-testing was conducted in central Honduras. An **Aedes aegypti** and **Culex spp**. panel of individual and mixed pools (n = 30) of adult mosquitoes, pupae, and larvae demonstrated 100% concordance in sensitivity (22/22) and 97% concordance in specificity (29/30) with one false positive. Field-testing of an **Aedes aegypti** and **Culex spp**. blind panel (n = 16) consisting of individual and mixed pools of adult mosquitoes, pupae, and larvae demonstrated 90% concordance in sensitivity (9/10) and 88% concordance in specificity (14/16).*

Introduction

The anticipation, prediction, identification, prevention, and control of vector-borne disease threats to military personnel are critical in all military operations. Real-time surveillance of mosquitoes and their respective immature stages allows rapid assessment of potential disease transmission risk and timely implementation of appropriate control measures. *Aedes aegypti* is the primary vector of dengue fever and yellow fever viruses therein representing a substantial threat for disease transmission to humans in many subtropical and tropical regions of the world (1). Dengue fever is the most significant mosquito-borne viral disease today. While malarial disease can be prevented by prophylaxis and yellow fever by immunization respectively, dengue

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fever prophylaxis does not exist and an approved vaccine is not anticipated in the near future. Currently, the only method of preventing infection with the dengue virus is vector avoidance.

Ae. aegypti is a peridomestic, diurnally active mosquito that prefers to breed in artificial containers near human habitations. Transmission of viruses to humans is by blood feeding females exclusively since males are non-biting. Vertical and possibly venereal transmission of dengue virus occurs by infected female to progeny (transovarian) (2, 3) and infected male to female during copulation, respectively (4). Therefore, while male mosquitoes do not directly infect humans they must be considered in the transmission cycle. In the absence of viremic hosts, these modes of transmission ensure survival of viruses in nature.

Control of disease transmission in endemic regions has become progressively more challenging as container-breeding mosquito habitat increases with exponentially increasing human populations and diminishing public resources for planning and controlling urban development (1). Depletion of public health resources has resulted in a lack of, or inefficient, mosquito control. Expanding global travel has exacerbated the problem by driving virus circulation in previously non-endemic regions thereby enhancing the potential for epidemics. Moreover, global warming influences local climatic patterns potentially making them more favorable for establishment and development of *Ae. aegypti* (5, 6, 7).

Efficacious surveillance of vector species, and their pathogens, is fundamental to the assessment of disease risk and time-critical implementation of appropriate transmission prevention measures and mosquito control. We describe here a real-time polymerase chain reaction (PCR) assay for sensitive and specific identification of *Ae. aegypti* and its respective life stages using field-deployable instrumentation.

Materials and Methods

Primer and probe design

Optimal probe and primer sequences were computed using Primer Express software according to the manufacturer's instructions (PE Applied Biosystems, Foster City, CA). Primer sequences were identified with Tm values 10 degrees less than that of the probe. The fluorescent reporter molecule at the 5' end of the TaqMan probe was 6-carboxy-fluorescin (FAM) and the quenching molecule was 6-carboxy-tetramethyl-rhodamine (TAMRA). Primers and probe olignucleotides were synthesized commercially (Synthetic Genetics, Rockville MD). Requests for sequences can be submitted through the corresponding author.

Assay optimizations

Preliminary assay optimization was performed on a LightCycler™ (Roche Molecular Biochemicals, Mannheim, Germany) and transferred to the "Ruggedized" Advanced Pathogen Identification Device (R.A.P.I.D.) [Idaho Technology Incorporated, Salt Lake City, UT, www.idahotech.com] using fluorogenic probe hydrolysis (TaqMan) based-PCR (8, 9). Assays were optimized with a proprietary buffer system (Idaho Technology Incorporated, Salt Lake City, Utah) and sensitivity and specificity validation testing completed.

Reaction conditions.

Assay optimizations and cross-reaction testing were conducted on the R.A.P.I.D. prior to sensitivity and specificity validation testing. Master mix reaction solution was prepared and 18 µl volumes dispensed into optical capillary tubes and 2 µl of DNA extract added from specimens and controls or 2 µl PCR grade water

for no template controls (NTC). Capillaries were placed in a tabletop centrifuge and spun for 2-3 seconds at 3000 rpm to drive the assay mixture to the bottom of the tube. Master mix components were 2X Quantitech Probe PCR Master Mix (Qiagen, Valencia, CA). Forward primer concentration was 0.30 µM, reverse primer 0.90 µM, and TaqMan probe 0.10 µM. A standardized RT-PCR thermal cycling protocol was established that consisted of an initial DNA denaturation at 94°C for 2 minutes and PCR for 45 cycles at 94°C for 0 seconds of template denaturation and 60°C for 20 seconds of combined annealing and primer extension. A single data point at the end of each annealing-extension cycle was collected and reported as TaqMan probe fluorescence released by 5'-nuclease activity during primer extension. Fluorimeter gains were set at 8-2-2 on channels 1, 2, 3 respectively. The criterion for a positive result was a significant increase in fluorescence over background levels as defined by an algorithm provided in the R.A.P.I.D analytical software (Roche Molecular Biochemicals, Indianapolis, IN).

Laboratory Evaluations of *Aedes aegypti* PCR Assays

Mosquito panels

Evaluations of the *Aedes* genetic assay for sensitivity and specificity were accomplished under controlled conditions at AFIERA. Lab evaluations were conducted on adult mosquitoes (*Ae. aegypti*, *Anopheles stephensi*, *Culex pipiens*, *Cx. quinquefasciatus*, *Ochlerotatus taeniorhynchus*), various pools of these species, and *Ae. aegypti* eggs provided by the Department of Virology, United States Army Research Institute of Infectious Diseases, Fort Detrick, Maryland (USAMRIID) (Table 1). Species identification and confirmation was accomplished by morphological examination and serological analyses by U.S. Army entomologists.

Mosquitoes were held in cardboard cages, provided a carbohydrate source (either apple slices or a gauze pad soaked in a 10% sucrose solution) and a water-soaked cotton pledget, and held at 26°C for 7 days. Mosquitoes were then killed by exposure to -20°C for 5-10 minutes and these placed into sterile 1.5-ml Eppendorf tubes and triturated in 750 µl TrRIzol-LS (Life Technologies, USA). Panels were established as shown in Table 2 labelled under code at USAMRIID, and shipped on dry ice to Molecular Epidemiology, AFIERA, Brooks AFB, San Antonio, Texas, for nucleic acid extraction and blind PCR analyses.

DNA preparation

Single adult mosquitoes and mosquito pools were placed in sterile 1.5 ml eppendorf tubes, homogenized with a sterile, blunted 1000 µl pipette tip in 200 µl of sterile water. Sample homogenates were spun for 60 seconds at 13,500 rpm on a tabletop centrifuge, and approximately 200 µl of supernatant were pipetted into the MagNAPure LC sample cartridge for processing. Nucleic acid was isolated using the MagNAPure LC System and MagNAPure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany) (10, 11). All post loading processing was completed in a closed system by automated robotics with preformatted reagents and a nucleic acid isolation matrix. Cell lysis and nucleic acid stabilization was completed with buffer containing guanidinium thiocyanate and proteinase K. Nucleic acid bound to the surface of magnetic glass particles was isolated from other cellular components by washing and eluting with a low-salt buffer. Nucleic acid extraction of mosquito eggs was by Trizol (Life Technologies, Rockville, MD) according to the manufacturer's instructions with the exception that sample homogenate was spun for 60 seconds at 13,500 rpm on a table-top centrifuge and 500 µl of supernatant exposed to the extraction process.

Field Evaluations of *Aedes aegypti* PCR Assays

Mosquito panels

The field site was central Honduras, 17-25 September 2002. Sampling was conducted in Comayagua and Tegucigalpa. Two teams of approximately 3-4 people each consisting of entomologists, physicians, public health professionals and technicians used battery-powered, hand-held aspirators to collect mosquitoes from the homes of consenting individuals, discarded tires and other structures. Immature mosquitoes were collected from various natural and man-made containers when present. For the field evaluations, all life stages, exclusive of eggs, were evaluated. Captured adult mosquitoes were temporarily held in storage tubes placed on dry ice (3 hours or less) and immature stages where held in "mosquito breeders" and returned to the field laboratory for processing. Additional specimens of pupae and larvae were collected and preserved in 95% ethyl alcohol for later identification and verification. United States Air Force entomologists identified and pooled captured and reared live adult mosquitoes, pupae, and larvae.

Specimens were pooled as *Ae. aegypti* alone, and in various combinations with *Culex* spp. Adult mosquitoes were placed in a freezer until they were rendered moribund, immediately transferred into 500 µl of Trizol, and then nucleic acid was extracted as described above. Larvae and pupae were placed directly into Trizol reagent prior to the extraction process. Optimized PCR assays described above were conducted using the R.A.P.I.D. Two experiments were conducted on the field-collected mosquitoes. In the first experiment, the R.A.P.I.D. operator had prior knowledge of the species composition in each prepared pool (Table 1). In the second experiment, the operator was provided mosquito pools as blind samples of unknown identity and composition (Table 2).

Results

Laboratory Evaluations

Sensitivity and specificity testing in laboratory evaluations showed the assay to be highly efficacious with excellent levels of detection for this species (Table 1). Laboratory testing of individual adult mosquitoes and mixed mosquito pools demonstrated 100% concordance in both *in vitro* sensitivity (6/6) and specificity (10/10) testing. Single adult *Ae. aegypti* were identified in pools of 100 non-*Aedes aegypti* mosquitoes, and the limit of detection of *Ae. aegypti* egg pools was five eggs. Because large mosquito pools are not technically practical with our current method of nucleic acid extraction, pools of greater than 100 were not evaluated in this study. Moreover, egg pool sizes of 5-10 exceed surveillance requirements therefore assay sensitivity was not optimized to a limit of detection of a single egg. Inhibition of PCR did not occur with Ae. aegypti-spiked pools of non-*Ae. aegypti* species.

Field Evaluations

Field-testing of the assay with a known panel consisting of individual and mixed pools of adult mosquitoes, pupae, and larvae demonstrated 100% *in vitro* sensitivity (22/22) and 97% specificity (29/30) with one false positive (Table 2). A single female *Culex* appeared to test positive after 40 PCR cycles (Ct = 40.52), but this likely was due to cross contamination in the laboratory since this specimen may have picked up some *Ae. aegypti* DNA when it was in combined storage before separation. Field-testing of the assay with a blind panel consisting of individual and mixed pools of adult mosquitoes, pupae, and larvae demonstrated 90% *in vitro* sensitivity (9/10) and 88% specificity (14/16) [Table 2]. One *Ae. aegypti* in a pool of 12 *Culex* produced a

negative result and a single *Culex* larva registered as a false positive. The *Culex* false positive (Ct = 39.03) error was likely due to species cross-contamination that occurred when the specimens were in storage. Whereas, multiple field-collected specimens at various stages of development would be tested *in situ*, sensitivity and specificity performance meets vector surveillance requirements.

Discussion

Rapid identification of both pathogens and their arthropod vectors is paramount for protecting military personnel (12). Likewise, surveillance of mosquitoes and their respective immature stages allows continued assessment of potential transmission risk and timely implementation of appropriate mosquito control measures. However, many military entomologists lack the taxonomic skills necessary to accurately identify vectors beyond the genus level. Public health personnel who are often tasked with conducting entomological surveillance generally are less experienced in species identification.

In the United States Air Force (USAF), arthropods, primarily mosquitoes and ticks, collected during routine surveillance are packaged and shipped to an out-of-area laboratory for identification by an entomologist with taxonomic skills. Although this approach is largely successful for getting specific identifications of potential vectors, the time involved for this process often conflicts with the requirement for rapid and specific identification to help in the prediction and prevention of vector-borne disease outbreaks. For example, the USAF primarily uses ovitraps to conduct base-level surveillance for *Aedes* (*Stegomyia*) mosquitoes and then rears the collected eggs to obtain adults for positive identification (13, 14, 15, 16, 17). However, under field conditions, especially in areas where disease transmission is active or where environmental conditions prohibit use of ovitraps (18), this method may not be practical. Identifying *Ae. (Stegomyia)* mosquitoes under field conditions also may not be practical when adults are not present, and identification of immature stages can prove challenging for untrained personnel. Moreover, there is an occasional requirement to conduct mosquito surveillance over a large geographical area or from a large number of locations that may involve the separation of the immature stages of *Ae. aegypti* and related species and/or the laboratory rearing of mosquitoes from positive ovitraps (19). Because of space and time requirements, substantial logistical problems can arise for such large-scale studies (20).

The *Ae. aegypti* assay described in this work clearly shows that both adult and immature specimens of this species can be accurately and rapidly identified by untrained personnel using the R.A.P.I.D. from both pure culture and mixed species pools. Our efforts have demonstrated the field utility and practicality of a rapid and accurate genomics-based vector identification capability. This methodology may offer a faster and more direct approach to identifying container-breeding *Aedes* species by eliminating the time consuming requirements or rearing adults from eggs collected in ovitraps. However, we have not yet fully evaluated the specificity of our assay on other mosquito taxa, and until this data is obtained we consider these data preliminary. Validation testing of assay specificity will remain an ongoing process as additional species of *Aedes* (*Stegomyia*) and other mosquito taxa become a part of our continually-expanding nucleic acid archive. We ultimately envision expanding this detection capability to include all of the principal vector species and pathogens of military importance.

PCR-based genetic assays are relatively simple and inexpensive to develop and use in both laboratory and field environments. Ultimately, they may offer a powerful tool for conducting surveillance of important vectors species without the requirement of basing identification on adult stages. Identifying mosquitoes can prove challenging for the untrained observer even with simplified diagnostic information (21). We believe that

our findings may have application for mosquito researchers and public health organizations requiring rapid identification of large numbers of samples, or diverse samples that may contain multiple vector species rather than using traditional time-consuming sorting and identification methods. Our assay system allows rapid, field identification of adult, larval, pupal and egg stages of *Aedes aegypti*.

Reference to trade name, vendor, proprietary product or specific equipment is not an endorsement, a guarantee or a warranty by the Department of the Defense, Army or Air Force, and does not imply an approval to the exclusion of other products or vendors that also may be suitable. The manuscript was cleared through the Technical Publication/Presentation Control Record, Brooks City-Base, TX for Open Publication, January, 2004. The conclusions and opinions expressed in this document are those of the authors. They do not reflect the official position of the U.S. Government, Department of Defense, Joint Program Office for Chemical and Biological Defense, U.S. Air Force, U.S. Army, or the Association of Military Surgeons of the United States.

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Table 1 Laboratory Evaluation of *Aedes aegypti* PCR assay using the LightCycler and R.A.P.I.D.

Sample Composition	PCR Results	Cycles (Ct)
1 Ae. aegypti	Positive	20.15
1 Ae. aegypti	Positive	20.88
1 Ochlerotatus taeniorhynchus	-	
1 Ochlerotatus taeniorhynchus	-	
2 Culex pipiens	-	
2 Culex pipiens	-	
2 Ae. aegypti (Trinidad strain)	Positive	26.93
2 Ae. aegypti (Trinidad strain)	Positive	26.39
2 Culex pipiens quinquefasciatus	-	
2 Culex pipiens quinquefasciatus	-	
2 Anopheles stephensi	-	
2 Anopheles stephensi	-	
24 Ae. aegypti (Trinidad strain) / 1 Ae. aegypti	Positive	25.73
24 Ae. aegypti (Trinidad strain) / 1 Ae. aegypti	Positive	26.43
24 Culex pipiens / 1 Ae. aegypti	Positive	34.18
24 Culex pipiens / 1 Ae. aegypti	Positive	33.61
24 Culex pipiens quinquefasciatus / 1 Ae.aegypti	Positive	33.95
24 Culex pipiens quinquefasciatus / 1 Ae.aegypti	Positive	34.52

24 Ochlerotatus taeniorhynchus / 1 Ae. aegypti	Positive	32.37
24 Ochlerotatus taeniorhynchus / 1 Ae. aegypti	Positive	32.29

Mosquito Pools: *Ae. aegypti* / Non-*Ae. aegypti*

1/50	Positive	28.94
1/50	Positive	28.72
1/50	Positive	30.96
1/75	Positive	33.64
1/75	Positive	33.21
1/100	Positive	33.51
1/100	Positive	33.91

***Ae. aegypti* Egg Pools**

100	Positive	30.15
50	Positive	32.96
10	Positive	34.7
5	Positive	30.68
1	Negative	

Table 2 Field Evaluation of *Aedes aegypti* PCR assay using the R.A.P.I.D.

Sample Composition: Known Panel	PCR Results	Cycles (Ct)
1 *Aedes aegypti* female	Positive	32.8
1 *Aedes aegypti* female	Positive	32.7
1 *Culex* female	-	
1 *Culex* female	Positive	40.52
2 *Aedes aegypti* females	Positive	29.88
2 *Aedes aegypti* females	Positive	34.72
2 *Aedes aegypti* males	Positive	32.05
15 *Aedes aegypti* male/female	Positive	29.7
1 *Aedes aegypti* female/12 *Culex*	Positive	30.91
1 *Aedes aegypti* female/12 *Culex*	Positive	32.48
1 Aedes aegypti	Positive	28.37
1 Aedes aegypti	Positive	28.97
1 *Aedes* larva	Positive	26.01
1 *Aedes* larva	Positive	27.31
1 *Aedes* larva	Positive	26.57
1 *Aedes* larva	Positive	25.97
1 *Aedes* pupa	Positive	25.85
1 *Aedes* pupa	Positive	25.94
1 *Aedes* pupa	Positive	25.92
1 *Aedes* pupa	Positive	26.48
1 *Culex* larva	-	
1 *Culex* larva	-	

Sample Composition: Known Panel	PCR Results	Cycles (Ct)
1 *Culex* larva	-	
1 *Culex* pupa	-	
1 *Culex* pupa	-	
1 *Aedes aegypti* larva/12 *Culex* larvae	Positive	33.00
1 *Aedes aegypti* larva/12 *Culex* larvae	Positive	38.21
1 *Aedes aegypti* larva/12 *Culex* larvae	Positive	30.60
1 *Aedes aegypti* larva/12 *Culex* larvae	Positive	31.35

Sample Composition: Blind Panel	PCR Results	Cycles (Ct)
1 Aedes aegypti	Positive	20.71
1 Aedes aegypti	Positive	24.24
1 *Aedes aegypti* larva	Positive	28.32
1 *Aedes aegypti* larva	Positive	26.91
1 *Aedes aegypti* larva	Positive	28.19
1 *Culex* larva	-	
1 *Culex* larva	-	
1 *Culex* larva	Positive	39.03
1 *Aedes aegypti* larva/3 *Culex* larvae	Positive	30.75
1 pupa unknown (presumed *Culex*)	-	
1 pupa unknown (presumed *Culex*)	-	
1 *Aedes aegypti* larva/12 *Culex* larvae w/debris	Positive	33.81
1 *Aedes aegypti* larva/12 *Culex* larvae w/debris	-	
1 *Aedes aegypti* larva/12 *Culex* larvae w/debris	Positive	38.60

Sample Composition: Blind Panel	**PCR Results**	**Cycles (Ct)**
12 *Culex* larvae w/ debris	-	
Debris only *Culex* container	-	
Debris only *Aedes aegypti* container	-	

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SYMPOSIA DISCUSSION - PAPER 22

Authors Name: Mr McAvin (US)

Discussor's Name: Dr Rios-Tejada (SP)

Question:
Can studies performed in Honduras can be extrapolated to other environments or sites?

Author's Reply:
Definitely not directly on site testing should be performed.

Authors Name: Mr McAvin (US)

Discussor's Name: Dr Rios-Tejada (SP)

Question:
When will serotype identification be ready and available on site?

Author's Reply:
It is ready and on the way to be adopted in a commercial way.

Field Evaluation of a Fluorogenic Probe-Based PCR Assay for Identification of a Visceral Leishmaniasis Gene Target

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ABSTRACT

*Visceral leishmaniasis (VL) is a potentially fatal disease caused primarily by **Leishmania donovani** complex species in Old World endemic regions. Pathogenesis requires a virulent factor transcribed by a gene family designated A2. An A2 sequence specific fluorogenic probe hydrolysis (TaqMan) PCR assay was developed on a field-deployable assay platform for real-time screening of the sand fly vector for VL causative agents. Laboratory-based assay optimization and specificity testing were conducted with total nucleic acid extracted from **L. donovani**-infected **Phlebotomus alexandri**, the primary vector. Cross-reactivity did not occur when tested against total nucleic acid extract of **L. tropica**-infected **P. sergenti, L. major**-infected **P. papatasi**, clinically relevant organisms, diverse viral and bacterial species, vector and human genomic DNA. Field evaluations were conducted in south central Iraq with sand fly pools screened by a previously established **Leishmania** universal PCR assay. The **Leishmania** universal assay was field-formatted (lyophilized) prior to deploying and upon field-evaluation against cold chain maintained wet reagents results were 98% (55/56) concordant. Of 86 **Leishmania**-positive sand fly pools, six were identified as A2 positive with a field-formatted visceral genotype specific assay.*

Introduction

The disease group leishmaniasises are caused by obligate intracellular parasites of the genus *Leishmania*. *Leishmania donovani* complex species are pathogenic agents of potentially fatal visceral leishmaniasis (VL) (1, 2). These protozoa exist as free-living promastigotes in the gut lumen of the sand fly vector (Old World genus *Phlebotomus*) and when transmitted to vertebrate host develop into amastigotes that proliferate within the macrophage phagolysosome. It has been shown that a *L. donovani* stage-specific gene family, designated A2, encodes protein required for amastigote development, which can ultimately be expressed in humans as VL (3, 4, 5). The leishmaniasises are also expressed in nonfatal forms, cutaneous and mucocutaneous. Old World visceral leishmaniasis, or kala-azar, is caused by *Leishmania donovani* complex species that include, *L. donovani* and *L. infantum*, and while *L. tropica* is typically a cutaneous leishmaniasis causative agent the virulence of this species can be manifested in the VL form of the disease (6).

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Iraq represents one of the most leishmaniasis prevalent regions globally (1). In the Nasiriyah area of south central Iraq the cutaneous forms of the disease are so common that cases usually go unreported (7). Clinicians in An Nasiriyah estimate nearly 1000 confirmed cases of VL each year for the past several years (7). Clinical laboratory methods of VL diagnoses are by microscopic identification of infected tissues, *in vitro* culture, or animal inoculation; identification of *Leishmania* DNA in infected tissue samples; and immunoassay (8). Immunochromatographic strip assays for the detection of antibodies to *L. donovani* in human serum are easily transported and require minimal training for use (9). An FDA approved kit is currently distributed to Iraqi clinicians through the World Health Organization and Coalition Provincial Authority, Baghdad, Iraq. Treatment of VL is an arduous process for the patient, requires specialized training for administration, and the expense untenable for many health care facilities (1, 2). Prevention is paramount in the control of the disease.

Surveillance of leishmaniasis causative agents requires deployable assays specific to pathogenic species and strains of *Leishmania*. Efficacious surveillance of VL causative agents allows timely assessments of transmission risk and time-critical implementation of personal protection measures and appropriate vector control responses. While the assay system described in the paper is not currently approved for clinical diagnostics, preliminary data suggest that it will prove a deployable method for rapid, sensitive, and specific identification of the visceral leishmaniasis causative genotype in sand flies. To validate the efficacy of our deployable system, field-formatted *Leishmania* universal (LEIS) and *Leishmania* visceral genotype specific (LVL) PCR assays were deployed to a visceral leishmaniasis endemic area in south central Iraq, September 2003. The study site was Tallil Air Base.

Materials and Methods

Primer and probe design

Visceral leishmaniasis genotype specific primer and probe sequences were designed by targeting A2 gene family loci and aligning homologous regions within *Leishmania donovani* complex species that excluded species from *L. tropica, L. major, L. aethiopica,* and *L. mexicana* complexes. Alignments were compared visually using the Clustal algorithm (10) in the MegAlign program of DNA Star software (Perkin Elmer, Norwalk, Conn.) [11]. Maximally conserved oligonucleotide sequences were chosen from *L. donovani* and *L. infantum* genomes downloaded from Genebank. *Leishmania tropica, L. major, L. aethiopica, and L. mexicana* complex species genomic sequences were aligned and visually evaluated to validate heterology with A2 primer and probe sequences. Genotype A2 specific primer and probe sequences can be requested through the corresponding author. The probe was a dual fluorogenic label design with a 5' reporter dye, 6-carboxyfluorescein (FAM), and 3' quencher dye, 6-carboxytetramethylrhodamine (TAMRA) [12].

Genotype A2 specific primers and probes sequence heterology with genomic sequences of closely related species through diverse genera were validated by BLAST database search (BLAST, Madison, Wisconsin) [13]. Melting temperatures were quantified and the absence of significant primer dimerizations and secondary structure (hairpin) formations were confirmed with PrimerExpress software (PE Applied Biosystems, Foster City, Calif.). Primers and probes were synthesized and quality control conducted commercially (Synthetic Genetics, San Diego, Calif.). Primers and probe were designated Leishmaniasis Visceral (LVL). A previously established *Leishmania* universal assay (LEIS) was obtained through collaboration with the Infectious Disease Clinic, Walter Reed Army Medical Center, District of Columbia and Walter Reed Army Institute for Research, Silver Spring, MD (14).

Sand Fly Pools

Over a five month period (April through August 2003) prior to this study, US Air Force and Army entomologists captured sand flies in battery powered light traps deployed at dusk within the Tallil Air Base perimeter at locations densely inhabited military personnel and uninhabited control locations. On the following morning, female sand flies were sorted into pools of 5-15, and preserved in ethanol. Total nucleic acid extract was prepared and screened for *Leishmania* spp by LightCycler-based PCR (Idaho Technology Incorporated, Salt Lake City, Utah; www.idahotech.com) (15) utilizing wet LEIS reagents. To evaluate the efficacy of the field-deployable assay system a blind test panels nucleic acid extracts was prepared from field-caught sand fly pools that had tested positive by LEIS wet reagent analyses (Table 1, 2).

DNA preparation

Total nucleic acid extracts were prepared with a commercially available, "off the shelf", thermo-stable, preformatted viral RNA purification kit, QIAamp viral RNA mini kit (QIAGEN, Valencia, California). Sand fly pools were homogenized in 560 µl AVL Buffer/Carrier RNA component of the kit with sterile, RNase free pestles and 1.5 ml tubes. Homogenate was cleared by centrifugation on a table-top centrifuge at 12,000 rpm for 60 seconds and total nucleic acid extract prepared following the manufacture's spin protocol. Each extract was suspended in 60 µl of elution buffer.

Reaction conditions

Wet *Leishmania* universal (LEIS) and visceral genotype specific (LVL) PCR assays were field-formatted, lyophilized, by a proprietary process [Idaho Technology Incorporated, Salt Lake City, Utah; www.idahotech.com]. Field analyses was conducted on field-durable, real-time, fluorimetric PCR/RT-PCR, thermocyling instrumentation - Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) [Idaho Technology Incorporated, Salt Lake City, Utah; www.idahotech.com] with lyophilized proprietary master mix components (Idaho Technology Incorporated, Salt Lake City, Utah). Master mix reaction solution was prepared by adding 40 µl of PCR grade water to lyophilized master mix reagent and dispensing18 µl volumes into optical capillary tubes. To each capillary, 2 µl of RNA extract added from specimens or 2 µl PCR grade water for no template controls (NTC). Capillaries were placed in tabletop centrifuge and spun for 2-3 seconds at 3000 rpm to drive the reaction mixture to the bottom of the capillary. *Leishmania* universal and LVL assay lyophilized master mixes contained *L. donovani* genomic DNA template and was prepared by adding 40 µl of PCR grade water.

A standardized PCR thermal cycling protocol was established that consisted of an initial DNA denaturation step at 94°C for 2 minutes, and PCR for 45 cycles at 94°C for 0 seconds of template denaturation and 60°C for 20 seconds of combined annealing and primer extension. A single data point at the end of each annealing-extension cycle was collected and reported as TaqMan probe fluorescence released by 5'-nuclease activity during primer extension. Fluorimeter gains were set at 8-2-2 on channels 1, 2, 3 respectively. Protocols for both LEIS and LVL assays were identical. The criterion for a positive result was a significant increase in fluorescence over background levels as defined by an algorithm provided in the R.A.P.I.D analytical software (Roche Molecular Biochemicals, Indianapolis, Ind.).

Results

Prior to field evaluation LVL wet PCR assay conditions were optimized to 0.10 pg (3 genomic equivalents) of *Leishmania donovani* genomic DNA (Table 1). Specificity testing was conducted with Old and New World *Leishmania* species, skin scrape preparations from ten cutaneous leishmaniasis patients, blood samples from eight malaria patients, clinically significant viral and bacterial species representing diverse species and strains, and human genomic DNA (Table 2). Testing with *Leishmania* infected vector species did not demonstrate cross-reactivity or inhibition of the PCR. All cross-reactivity test results displayed no detectable fluorescence above background with the exception of *Streptococcus equi*, a critical threshold slightly above background fluorescence (CT = 42) was observed upon testing of a single sample. Fluorescence was not at a level above background to consider the *Streptococcus equi* as a cross-reacting species but validation testing is planned. Laboratory-based optimization and specificity testing were conducted with LVL wet reagents on RAPID instrumentation. Field-formatted LEIS and LVL assays were deployed for evaluation. The LEIS assay when tested against cold chain maintained wet reagents results were 98% (55/56) concordant (Table 3). Of 86 *Leishmania*-positive sand fly pools, six were A2 positive (Table 4).

Discussion

In mid-July 2003 a team of US Air Force and US Army entomologists conducting *Leishmania* surveillance at Tallil Air Base, south central Iraq, expressed concern of a potential leishmaniasis outbreak situation. In response, we designed visceral leishmaniasis (VL) pathogen genotype specific PCR assay (LVL) primers and probe and conducted assay optimization and cross-reactivity testing through a joint AFIOH, WRAIR, USAMRIID, CHPPM-W effort. Field-formatting (lyophilization) of LVL and *Leishmania* universal (LEIS) assays was conducted by a corporate partner, Idaho Technology Incorporated, Salt Lake City, Utah. By late August, we deployed a developmental phase surveillance system for screening sand flies for *Leishmania* spp and identification of VL causative agents. Field-formatted master mix reagents and positive control were easily transported, field-sustainable, and only required hydration and addition of sample template prior to PCR (16, 17, 18). Wet and lyophilized LEIS assay results were concordant in blind testing and the LVL assay showed that vector-borne pathogens for VL were a significant threat.

These data supported force protection measures previously implemented by the entomologist team – stringent personal protection and vector control. Preliminary reservoir surveillance data of the primary reservoirs, rodents and canids (dogs and foxes), indicate that rodents are significant in the *Leishmania* transmission cycle while canids are not. Studies are planned to determine if rodent control will break the transmission cycle.

Additional testing of the LVL assay is planned to determine the efficacy of A2 gene family derived primer and probe sequences as a universal VL genotype target site. That *L. donovani* promastigote-to-amastigote development, and VL pathogenesis, requires an A2 gene family encoded factor defines this protein as fundamental to the life cycle of *L. donavani* and pathogenesis of VL. This suggests conservation in A2 gene family sequence homology across VL causative agents. Continued LVL specificity testing with diverse strains of *L. donovani*, *L. infantum*, and *L. archibaldi* as well as New World visceral leishmaniasis causative agent, *L. chagasi*, is planned. Moreover, testing with *L. tropica* strains implicated as causative agents in the visceral form of leishmaniasis will yield valuable LVL specificity data and potentially provide insight into the pathogenesis of the leishmaniasises.

The LEIS and LVL field-formatted real-time PCR assays have potential as an aid in human diagnostics. The deployable assay system provides an advantage over conventional diagnostic instrumentation especially in areas were clinical laboratory facilities are not available. Immunochromatographic strip assays provide deployable testing capability however require refrigeration. Moreover, immunoassays can potentially produce false negative results in immunologically immature or immunocompromised leishmaniasis patients and false

positive results in individuals whom have developed antileishmanial antibody in response to a previous infection (19).

This study demonstrated the operational utility of a deployable DNA probe-based assay platform for the surveillance of visceral leishmaniasis causative agents in sand flies and promising aid to clinical diagnostics. The assay system may yet have an additional operational application in *Leishmania* surveillance as rotating coalition forces return from southwest Asia to non-endemic regions.

Reference to trade name, vendor, proprietary product or specific equipment is not an endorsement, a guarantee or a warranty by the Department of the Defense, Army or Air Force, and does not imply an approval to the exclusion of other products or vendors that also may be suitable. The conclusions and opinions expressed in this document are those of the authors. They do not reflect the official position of the U.S. Government, Department of Defense, Joint Program Office for Chemical and Biological Defense, U.S. Air Force, U.S. Army, or the Association of Military Surgeons of the United States.

Acknowledgments

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Table 1 LVL PCR assay sensitivity

Genomic Quantity	Genomic Equivalents	CT
L. donovani 1.00 ng	2.90E+04	23.32
L. donovani 1.00 ng	2.90E+04	22.91
L. donovani 0.10 ng	2.90E+03	26.84
L. donovani 0.10 ng	2.90E+03	26.08
L. donovani 0.01 ng	2.90E+02	29.57
L. donovani 0.01 ng	2.90E+02	30.08
L. donovani 1.00 pg	2.90E+01	33
L. donovani 1.00 pg	2.90E+01	33.6
L. donovani 0.10 pg	2.90E+00	38.47
L. donovani 0.10 pg	2.90E+00	35.6
L. donovani 0.01 pg	0.00E+00	0
L. donovani 0.01 pg	0.00E+00	0

CT: critical threshold

Table 2 LVL PCR assay specificity

Species	LVL
Leishmania donovani	+
Leishmania tropica	-
Leishmania major	-
Leishmania mexicana	-
Leishmania braziliensis	-
Leishmania guyanensis	-
Leishmania amazonensis	-
Cutaneous leishmaniasis CS1 - CS10	-
Malaria Vivax CS1 - CS8	-
West Nile Virus 613ca	-
West Nile Virus 1278	-
Dengue virus serotype 1 CS1 - CS4	-
Dengue virus serotype 2 CS1 -CS6	-
Hanta virus	-
Leptospira borgpeternenii	-
Leptospira interrogans	-
Leptospira biflexia	-
Leptospira icterohaemmorrhagiae	-
Leptospira patoc-1	-
Rickettsia rickettsia	-
Ehrlichia chaffeensis pD2	-

Table 2 LVL PCR assay specificity (continued)

Species	LVL
Streptococcus . pyogenes	-
Staphylococcus agalactica	-
Staphylococcus. aureus	-
Neisseria meningitidis	-
Salmonella typhi	-
Brucella melitensis	-
E. coli O157	-
Homo sapien genomic DNA	-

Table 3 Conventional vs field-formatted LEIS PCR assay results

TAML Sample ID	Conventional LEIS PCR Assay Sreening (CT)	Conventional LEIS PCR Assay Confirmation (CT)	Field-Formatted LEIS PCR Assay Screening (CT)
888-001f			
1117-001f	29.83	29.98-29.13	28.91
1094-001aa			
1094-001c			
1120-001a	29.24	33.49-35.38	36.33
1121-001b	23.09	27.06-23.60	21.60
1094-001d			
1124-001c	27.30	28.56-27.97	25.96
1101-001b			
1129-001n			
1125-001b	21.09	31.34-19.85	18.56
1129-001d	27.08	26.11-26.99	24.83
1129-001x	27.13	26.35-26.48	24.35
1129-001o			
1129-001y	26.50	26.34-25.18	24.80
1129-001r			
1135-001c			
1139-001n	27.71	26.56-26.98	26.06
1220-001a	28.96	31.64-30.91	31.56
786-001c	28.46	29.53-28.25	27.70

786-001f	28.14	29.87-27.84	26.58
1135-001d			
789-001e	25.41	23.83-24.62	23.04
1139-001g			
1139-001h			
789-001l	24.93	25.63-25.59	23.26
1178-001d			
1214-001c	24.07	24.47-24.49	22.79
1214-001d	27.45	26.78-25.45	24.70
1178-001e			
1219-001a	23.31	20.30-21.45	18.56
1178-001f			
1194-001a			
1240-001a	22.12	21.52-23.16	19.91
1241-001a	28.04	27.49-27.70	24.36
1242-001a	24.14	23.43-24.21	21.92
1195-001a			
1225-001a			
1245-001c	29.28	29.48-30.90	28.94
1225-001b			
1248-001d	28.94	27.27-26.72	25.51
1251-001b	29.00	29.58-29.07	27.77
1238-001a			

1238-001b			
1252-001a	22.32	25.18-22.68	21.47
1268-001a			
1268-001d			
1252-001c	27.70	26.93-26.83	26.35
1255-001a	25.72	25.87-29.45	22.33
786-001e			
789-001a			
888-001d			35.20
888-001e			
1268-001b	18.66	20.42-18.50	17.25
1268-001c	22.63	22.31-23.63	19.89
1272-001a	22.11	22.00-21.74	19.35

CT: critical threshold

Table 4 Conventional LEIS vs field-formatted LVL PCR assay results

TAML Sample ID	Conventional LEIS PCR Assay Screening (CT)	Conventional LEIS PCR Assay Confirmation (CT)	Field-Formatted LVL PCR Assay Screening (CT)
1019-001c	31.79	32.9->36	0
754-001a	>36	>36->36	0
756-001c	>36	33.01->36	0
984-001a	>36	>36	0
901-001c	33.91	>36->36	0
901-001l	28.73	31.08-30.54	0
965-001a	24.21	27.03-26.38	0
965-001e	25.45	26.23-26.11	0
965-001g	31.03	32.38-32.01	0
965-001j	25.57	27.93-26.11	0
965-001k	30.29	32.32-32.23	0
1007-001d	26.47	27.04-26.89	0
1032-001g	>36	>36->36	0
1043-001b	24.71	24.88-24.39	0
1043-001c	33.10	33.02-32.68	0
1044-001a	21.59	21.78-21.57	0
1045-001c	28.57	29.76-29.45	0
1050-001a	23.30	25.86-25.08	0
1051-001c	24.82	26.6-26.27	0
1051-001d	23.31	25.16-24.88	0

1057-001a	28.31	27.54-27.72	0
1061-001a	28.86	29.29-29.71	0
1061-001b	>36	34.11->36	32.26
1061-001f	33.49	31.99-33.28	0
1071-001a	28.80	28.49-29.11	0
1074-001a	33.33	>36-Neg	0
1075-001b	32.05	28.41-Neg	0
1078-001b	29.57	>36->36	0
1079-001e	32.34	33.07-33.17	0
1097-001a	23.15	22.80-22.86	26.08
1103-001a	31.82	>36-35.7	0
787-001d	31.83	31.78-32.50	0
787-001e	>36	19.66-20.01	0
787-001g	19.47	26.16-26.02	0
788-001a	25.56	>36->36	0
884-001d	>36	>36->36	0
884-001f	34.26	20.84-20.61	0
884-001g	20.51	21.12-21.88	25.42
950-001p	27.93	28.35-27.75	0
1107-001a	28.03	28.47-28.42	0
1112-001b	26.63	26.87-27.55	0
1109-001c	25.86	27.01-26.28	0
808-001a	29.00	28.74-29.14	36.91
811-001a	25.18	25.47-25.57	0

812-001a	28.22	28.30-27.80	0
817-001a	23.78	23.73-24.51	0
817-001b	>36.00	35.72->36	0
819-001c	33.56	>36-Neg	0
1122-001c	30.10	29.18-31.43	0
1129-001q	35.69	33.72-33.36	0
1129-001v	34.18	>36-34.28	0
1139-001L	34.63	33.08-32.82	0
1141-001d	30.70	28.91-29.95	32.8
1141-001h	31.62	29.54-29.04	0
1172-001a	35.14	33.33-33.29	0
1174-001b	33.00	32.90-33.02	0
1180-001a	33.42	32.45-34.29	0
1220-001b	34.23	34.07-33.96	0
789-001b	30.43	30.62-30.47	0
789-001j	31.61	31.89-31.56	0
1214-001b	34.08	35.19-33.81	0
1214-001e	32.45	31.94-32.09	0
1214-001h	30.11	29.45-29.32	0
1214-001j	30.90	31.09-31.24	0
1216-001c	30.31	31.44-30.48	34.43
1238-001d	30.65	30.83-30.56	0
1239-001b	31.97	31.90-31.83	0
1239-001c	32.15	32.13-32.39	0

1243-001a	>36	35.48-32.15	0
1245-001e	33.01	32.82-35.56	0
1247-001a	>36	34.18->36	0
1248-001e	31.87	32.85-31.99	0
1249-001b	32.67	32.90-33.08	0
1250-001d	30.34	30.03-30.32	0
1251-001a	32.76	33.03-34.30	0
1251-001c	31.94	31.76-31.83	0
1253-001b	35.76	32.85-33.54	0
1253-001e	>36	33.97-35.78	0
1254-001a	>36	34.11-34.15	0
1257-001a	32.69	32.03-32.20	0
1268-001c	22.63	22.31-23.63	0
1272-001a	22.11	22.00-21.74	0

CT: critical threshold

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SYMPOSIA DISCUSSION - PAPER 23

Authors Name: Mr McAvin (US)

Discussor's Name: Capt (USN) Campbell (US)

Question:
There are published reports of the "Visceralizing" Cutaneous Leishmaniasis (L. Tropica) in troops returning from the first Persian Gulf War.

Given that you have shown your visceral leishmaniasis probe to specifically not recognize L. Tropica, are you concerned that your probe might miss (not detect) L. Tropica that may be capable of causing the potentially lethal, visceral form of the disease?

Author's Reply:
Yes specimens have been requested from WRAIR to determine if the LVL assay is capable of identifying viserotropic L. Tropica.

Diagnostic Biodosimetry Response for Radiation Disasters: Current Research and Service Activities at AFRRI

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ABSTRACT

Diagnostic Biodosimetry Response for Radiation Disasters Using Advanced Molecular Cytogenetic, Molecular Biology, and a Biodosimetry Assessment Tool: Current Research and Service Activities at the Armed Forces Radiobiology Research Institute

This paper addresses the importance of diagnostic radiation dose assessment to help develop a treatment strategy within days of a catastrophe. The long-range goals of the Armed Forces Radiobiology Research Institute (AFRRI) Biological Dosimetry Team are to develop validated radiation biodosimeters and to equip medical personnel with diagnostic information (clinical signs and symptoms, physical dosimetry, etc.) germane to the medical management of human radiation casualties. Our specific objectives are (a) to establish definitive, rapid, high-throughput clinical bioassays for radiation dose assessments, (b) to develop complementary triage-type radiation dose assessment bioassays, and (c) to transition the Biodosimetry Assessment Tool (BAT) software program to facilitate the collection, integration, and arching of biodosimetry data to support medical treatment decisions of radiation-exposed individuals.

The experimental approach involves three steps: (a) to establish a "reach-back reference laboratory" that uses conventional bioassays for definitive analyses of biological samples; (b) to develop a validated and forward-deployable biological dosimetry capability for rapid radiation dose assessment, with an emphasis on the use of molecular biology-based diagnostic platforms; and (c) to integrate the biodosimetry data in a suitable software platform to assist in medical management, for example, BAT software.

AFRRI researchers established the conventional lymphocyte metaphase-spread dicentric assay in accordance with international harmonized protocols and have been applying it in order to estimate radiation doses in several overexposure accidents. The researchers seek to validate a novel interphase, cell-based cytological bioassay that detects cells with chromosomal-type aberrations and radiation-responsive molecular biomarkers (e.g., gene expression, protein) and to perfect it for rapid radiation dose assessment applications. The BAT software program was released at the AFRRI website (www.afrri.usuhs.mil) in June 2002. Designed primarily for prompt use after a radiation incident, the user-friendly program facilitates collection, integration, and archiving of data obtained from exposed persons. Data collected in templates, using the Microsoft Windows-compatible, user-friendly software program, are compared with established radiation dose responses obtained from the literature to provide multiparameter dose assessment. The program

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archives additional clinical information (e.g., extent of contamination, wounds, infection) that is useful for casualty management, and it displays useful information in a concise format. The program is designed for both civilian and military applications, the latter illustrated by fulfilling the requirements of proposed NATO STANAG 2474 NBC/MED "Determination and Recording of Ionizing Radiation Exposure for Medical Purposes."

Our research and service efforts contribute to an improved diagnostic responses to mass casualty situations and enhance force protection and survivability in adverse ionizing radiation mission environments.

1.0 INTRODUCTION

Mettler [2002] reviewed potential radiation exposure scenarios, which included detonation of nuclear weapons, terrorist attacks on nuclear reactors, and dispersal of radioactive substances with the use of conventional explosives, resulting in mass casualties. These disasters can result in different radiation exposure types: whole body, localized or partial body, internal contamination, external contamination, and contaminated burns and wounds. Strategies for triage and for the evacuation of the injured, contaminated, and noncontaminated casualties are proposed herein. The onset, nature, severity, and duration of clinical symptoms following radiation exposure are determined primarily by the casualty's absorbed dose but are also influenced by the radiation field and quality, the dose rate, and the individual's inherent radiosensitivity and general medical health status. In reaction to prompt total-body ionizing radiation with a dose range of 0.5 to 30 Gy (photons), the typical symptoms of radiation exposure in humans include nausea, vomiting, diarrhea, and peripheral blood lymphocyte depletion [Anno 1989]. The duration of initial or prodromal symptoms and the latent phase of radiation syndrome is anywhere from 1 h to 2 weeks. Without appropriate medical care, the median lethal dose of radiation, the $LD_{50/60}$ (the dose that kills 50% of the exposed population within 60 days after exposure), is estimated to be 4.5 Gy [Mole 1984]. However, the likelihood of survival can be significantly increased with appropriate aggressive medical intervention and care [Anno 2003].

Nonavailability or inaccurate initial dose estimates, within hours to weeks after exposure, could result in suboptimal medical intervention. In all potential radiation exposure scenarios, it is unlikely that physical dosimeters will be available for dose assessment to aid clinical management of mass casualties. For early treatment of radiation victims, it is recommended that medical personnel rely heavily on clinical signs and biological dose assessments [Goans 1997]. However, early dose estimates may be required in radiation disasters that involve a large number of victims and a finite amount of medical resources available to responders and healthcare providers.

The focus of present research is based on the following factors.

- Radiation dose to the exposed individual is the primary determinant of the nature, onset, severity, and duration of acute radiation syndrome (ARS).
- Early diagnostic estimation of the absorbed dose is essential for effective clinical management.
- Medical personnel rely heavily on clinical signs and biological assessments of radiation exposure for clinical treatment.
- With appropriate medical and intensive care, the likelihood of near term survival can be increased significantly.

In all potential radiation disasters, a single population is likely to encounter a number of complex radiation exposure scenarios, including different dose ranges and dose rates. Therefore, a single biodosimetry assay

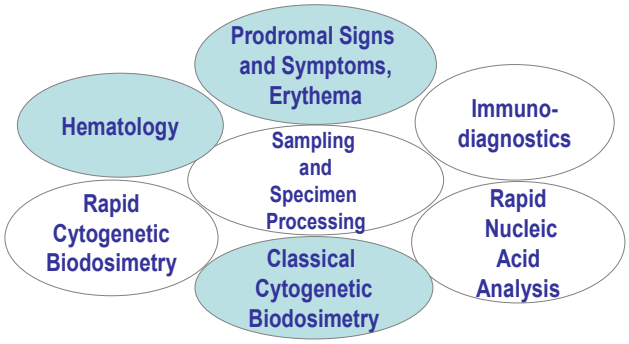
cannot fully address the biodosimetry requirement in complex exposure scenarios. Triage, clinical, and definitive radiation biodosimetry will all require multiple bioassays. Table 1, adapted from AFRRI's Medical Management of Radiological Casualties Handbook [AFRRI 2003], suggests validated biodosimetry methods for different dose ranges, expected prodromal effects, manifest symptoms, and survival expectancies.

Table 1: Proposed validated biodosimetry methods for different dose ranges, expected prodromal effects, manifest symptoms, and survival expectancies.

Dose range (Gy)	Proposed validated biodosimetry methods	Prodromal effects	Manifest symptoms	Survival expectancy
0.1 – 1.0	Dicentric/premature chromosome condensation (PCC)	None to mild (from 3 h to 48 h)	None to slight decrease in blood count	Almost certain
1.0 – 3.5	Lymphocyte depletion kinetics/dicentric/PCC	Mild to moderate (from 1 h to 48 h)	Mild to severe bone marrow damage	0 to 10% death
3.5 – 7.5	Lymphocyte depletion kinetics/PCC	Severe (from 1 h to 48 h)	Pancytopenia, mild to moderate GI damage	10 to 100% death (within 2 to 6 weeks)
7.5 – 10.0	Lymphocyte depletion kinetics/PCC	Severe (from <1 h to 48 h)	Combined BM and GI damage	90 to 100 % death (within 1 – 3 weeks)
>10.0	PCC	Severe (from minutes to <48 h)	GI, neurological and cardiovascular damage	100% death (within 2 to 12 days)

At AFRRI, we are developing a multifaceted and integrated biodosimetry system to fully address the need for triage, based on early physical assessments, bioindicators, and biological assessments, in order to aid clinical management of radiation accident victims (Figure 1). This system will help differentiate between exposed and nonexposed but concerned individuals. Our long-range goal is to develop and integrate a battery of validated radiation bioassays to equip medical personnel with diagnostic information (clinical signs and symptoms, physical and biological dosimetry, etc.) germane to the medical management of human radiation casualties. Our specific objectives are to (a) establish definitive, rapid, high-throughput clinical bioassays for radiation dose assessments; (b) develop, for triage purposes, complementary triage-type radiation dose assessment bioassays, such as molecular-biology based forward-deployable diagnostic platforms; and (c) transfer the Biodosimetry Assessment Tool (BAT) software program and complementary tools to healthcare professionals to facilitate the collection, integration, and archival of relevant biodosimetry information.

Figure 1. Illustration of an integrated and multiparameter diagnostic biodosimetry system.



2.0 DEFINITIVE RAPID, HIGH-THROUGHPUT CLINICAL BIOASSAYS

2.1 The Dicentric Assay

Radiation exposure induces many types of chromosomal aberrations in the exposed individual's peripheral blood lymphocytes. The presence of dicentrics, a chromosomal structural aberration, in an individual's peripheral blood lymphocytes indicates radiation exposure. Dicentrics are considered relatively radiation specific; only a few chemicals are known to interfere with the assay. Low background levels (about 1 dicentric in 2000 cells), high sensitivity (a threshold dose of 0.05 Gy), and known dose dependency of up to 5 Gy (for photons) make this assay robust and a "gold standard" biodosimetry method. This cytogenetic chromosome aberration bioassay is a thoroughly investigated biodosimetry method. The dicentric assay is conventionally used to provide definitive radiation dose assessment. Because exposure of human peripheral blood lymphocytes (HPBL) *in vitro* and *in vivo* produces similar levels of dicentrics per unit dose, dose estimates to an exposed individual can be made by comparing the observed frequencies of dicentrics to an *in vitro* generated dose-effect calibration curve [IAEA 2001].

2.1.1 Reference Cytogenetic Biodosimetry Laboratory

AFRRI supports the U.S. Department of Defense's medical readiness by providing a limited cytogenetic biodosimetry service capability for radiation dose assessment conforming to international guidelines following the establishment of "a reach-back" cytogenetic biodosimetry laboratory. Blood samples (10 to 15 ml) are collected from the exposed individuals as soon as practical, generally 1-day after exposure, and are transported to the laboratory where lymphocytes are isolated from whole blood and stimulated to grow in culture, metaphase spreads are harvested, and chromosome aberration analyses are performed using internationally accepted laboratory protocols [IAEA 2001].

The blood collection procedure for cytogenetic biodosimetry is described in AFRRI's Medical Management of Radiological Casualties Handbook [AFRRI 2003]. This medical management doctrine can be downloaded from AFRRI's website, www.afrri.usuhs.mil. Since 2000, AFRRI's cytogenetic laboratory has analyzed more than 15 cases from radiation incidents and accidents, using dose-response calibration curves in HPBL [Prasanna 2002a] by dicentric assay, and 12 cases from a radiological accident that occurred in Thailand in February 2002 using the Rapid Interphase Chromosome Aberration (RICA) assay [Boreham personal communication].

2.1.2 International Efforts on Cytogenetic Biodosimetry Method Harmonization

The biodosimetry scientific community realized the need to harmonize the cytogenetic dosimetric methodology because there is no universally adopted laboratory protocol and important variations occur between the laboratories, often influencing the quality of results. AFRRI scientists are involved in these international efforts to harmonize the biodosimetry cytogenetic methods to address this problem. The International Atomic Energy Agency (IAEA) published a technical manual, involving the efforts of AFRRI scientists, on cytogenetic biodosimetry that provides a harmonized methodology for various cytogenetic assays. This manual [IAEA 2001] provides information necessary for selecting and implementing, in a standardized manner, the appropriate cytogenetic method to ensure accurate dose assessments following an accidental exposure to ionizing radiation.

An International Organization for Standardization (ISO) working group, comprised of 13 scientists from 11 countries and an IAEA representative, was established to standardize biological dosimetry by cytogenetics. Under the auspices of the ISO, regulatory compliance and validation efforts are being made for the dicentric assay. The scope and structure of the working draft, ISO TC-85/SC-2, Radiation Protection – Performance Criteria for Service Laboratories Performing Biological Dosimetry, provides the guidelines for conducting the biological dosimetry by cytogenetics [Voisin 2002]. The "reach-back" cytogenetic biodosimetry laboratory in AFRRI is implementing good laboratory procedures (GLP) for quality control and quality assurance.

2.2 Premature Chromosome Condensation (PCC) Assay

Conventional metaphase-spread chromosome-aberration biodosimetry techniques are robust, but they are laborious, time-consuming, and, more importantly, require an *in vitro* stimulation of resting HPBL to cause proliferation. For potential high-dose irradiation above the median lethal dose, such as in a radiation disaster, it is expected that radiation-induced cell death and delay in cell cycle progression into mitosis will interfere with dose estimation [Prasanna 2002b]. In addition, high-dose radiation accident victims will also suffer from lymphopenia; therefore, few cells will be available for cytogenetic studies. In order to overcome this limitation, quantitative analysis of radiation-induced damage may be performed using resting HPBL in lieu of metaphase spreads. Use of interphase cytological assays, such as the PCC assay, could eliminate these inherent problems associated with the use of metaphase-spread cytogenetic assays. The PCC assay is useful to determine exposure to low doses as well as to life-threatening acute high doses of low-LET (linear energy transfer) [Prasanna 1997, 2000] and high-LET radiation [Prasanna 1997]. Moreover the PCC assay can discriminate between total- and partial-body exposures [Blakely 1995].

It was shown that PCC can be induced in resting HPBL through signal transduction mechanisms by a simple incubation of cells in a culture medium containing a protein phosphatase inhibitor, okadaic acid, mitosis-promoting factor $p34^{cdc2}$/cyclin B kinase [Prasanna 2000]. The interphase-based rapid interphase chromosome aberration (RICA) assay, is a simple alternative to the metaphase-spread based dicentric assay. In RICA assay, damage involving specific chromosomes is analyzed in chemically induced PCC spreads after fluorescence *in situ* hybridization (FISH) with specific whole-chromosome DNA hybridization probes (Prasanna and Blakely, international patent pending). In the RICA assay, the cells that display two chromosome spots are considered normal and cells with more than two chromosome spots are considered aberrant. The frequency of aberrant cells [Prasanna 2000] and the number of aberrations per cell [Prasanna 2002b] are shown to increase with radiation dose over a broad dose range encompassing those well above the median lethal dose. This invention in cytogenetics has wide applications across biotechnology and biomedical fields.

Recently, the RICA assay was used to assess chromosome damage in individuals accidentally exposed to gamma radiation in Samutprakarn, Thailand, in studies designed to validate this method for assessing radiation dose to human subjects. This study used a cohort of several individuals accidentally exposed to gamma rays from an unshielded ^{60}Co source; they received acute, chronic, or fractionated exposures. The frequencies of aberrant chromosome 1 showed a good correlation with clinical symptoms of acute radiation syndrome: mainly nausea, vomiting, severe headache, fever, and depletion in white blood cell counts [Boreham submitted].

2.3 Cytogenetics in Mass Casualty Scenarios

Cytogenetic biological dosimetry can also make valuable contributions to the medical management of patients in the early period after a radiation disaster, where a rapid confirmation of dose is required. At such a time, all that is needed is a rapid triage of casualties, based on approximate dose estimation using biological and clinical endpoints, rather than precise dose estimations for a vast number of individuals. Recently, a consensus document generated by the Dosimetry Sub Panel of the Radiological/Nuclear Threat Countermeasures Work Group of the Office of Science and Technology Policy (OSTP, U.S. Homeland Security Council) recommended that sufficient supplies for cytogenetic biodosimetry procedures be available in a national stockpile for emergency radiation disaster management. AFRRI scientists contributed to the document.

The utility of cytogenetic assays to assess health risks and to guide medical treatment decisions was demonstrated in several radiation accidents involving mass casualties, such as those referred to as Chernobyl, Goiania, and Tokaimura. Table 2 summarizes the information on the use of cytogenetic methods in radiation accidents. Estimated doses using cytogenetic methods correlate well with the severity of acute radiation syndrome [Sevan'kaev 2000]. In the Chernobyl, Russia, accident, an approximate dosimetry was achieved by rapid preliminary examination of 50 lymphocyte metaphases per person for several individuals [Pyatkin 1989]. Ramalho [1991] investigated 129 exposed or potentially exposed individuals from the Goiania, Brazil, accident cohort immediately after the radiological emergency. Dose estimates exceeded 1 Gy for 21 subjects from this cohort and 4 Gy for 8 individuals. More recently, dose estimation was done using the dicentric and PCC assays in the Tokaimura, Japan, criticality accident in 3 severely exposed workers [Kanda 2002, Hayata 2001] and 43 resident workers [Sasaki 2001]. These radiation accidents highlight the importance of the cytogenetic methods in early dose assessment after a radiological event and demonstrate their ability to influence medical treatment decisions.

Recently, it was suggested that the dicentric assay could be adapted for the triage of mass casualties [Lloyd 2000, Voisin 2001, Prasanna 2003]. Lloyd [2000] described an *in vivo* simulation of an accident with mass casualties receiving whole- or partial-body irradiation in the 0- to 8-Gy range. Faced with an urgent need for rapid results, clinical triage was accomplished by scoring as low as 20 metaphase spreads per subject, compared with the typical 500 to 1000 spreads scored in routine analyses for estimating dose. However, Lloyd [2000] suggested increasing the analyses to 50 metaphase spreads when there is disagreement with the initial assessment or when there is evidence of significant inhomogeneous exposure. After the initial results are communicated to the treating physician, additional scoring is recommended to resolve potential conflicts in dose assessment and, in the case of high doses, to assist physicians considering marrow-stem-cell transfusions to mitigate bone marrow ablation. Using the dicentric assay in this triage mode, a reasonable throughput of 500 or more samples per week per laboratory is achievable [Prasanna 2003].

Table 2. Cytogenetic assays in radiation mass casualties.

Accident location	Year of accident	Number of people exposed	Number of samples analyzed using cytogenetic methods		Reference
			Dicentrics	PCC*#	
Chernobyl, Russia	1986	~ 116,000	158	NA	Sevan'kaev [2000]
Goiania, Brazil	1987	~ 250	129	NA	Ramalho [1991]
Tokaimura, Japan	1999	3** Unknown***	1** 43***	3**	Kanda [2002] Hayata [2001] Sasaki [2001]

*#Premature chromosome condensation assay, **criticality exposed workers, ***resident and other workers, #the PCC assay permits dose assessment above 4 Gy.

2.3.1 Cytogenetic Laboratory Automation and High-Throughput Analysis

The use of commercially available off-the-shelf instruments, such as liquid-handling robotic devices, automated metaphase finders, and multiple satellite chromosome aberration analysis stations, is recommended for triaging radiation mass casualties for a rapid turnover of results. Efforts are under way at AFRRI to automate, for military and civilian applications, assays based on chromosome damage. The automated cytogenetic biodosimetry process is outlined in Table 3. The throughput of the cytogenetic laboratory can be increased by using liquid-handling robotic devices for handling blood and for isolating lymphocytes, by using microprocessor-controlled multi-pipettes for transferring reagents, by using automated metaphase harvesters and spreaders with modules for 20 to 50 slides for simultaneous staining, and by using automated instruments for DNA hybridization of centromere/whole-chromosome-specific probes and immunoenzymatic chromosome painting. These methods will also ensure quality control and quality assurance under the good laboratory practice environment for conducting assays.

Specialized cytogenetic laboratories ususally rely on automated metaphase-finder systems for locating suitable chromosome spreads for analysis [Lloyd 1990, Prasanna 2002a]. A typical metaphase finder consists of a standard binocular microscope equipped with a stage that accommodates a few hundred slides and motorized x- y- and z-axis computer-controlled positioning with specially adapted autofocus capabilities. The system includes specialized software utilities (Loats Associates Inc., Westminster, MD) that permit user control of image recognition parameters and relocation of metaphase spreads on the metaphase finder as well as multiple satellite chromosome aberration analysis stations for rapid analyses by multiple scorers to increase throughput.

Table 3. Components of automated cytogenetic radiation bioassay.

Bar-coding and tracking of reagents, sample processing, scoring, and analysis	Metaphase spreader
Automated blood lymphocyte isolation system	Automated slide stainer and cover-slipping
Automated blood lymphocyte culturing system	Automated microscope - metaphase finder
Automated metaphase harvester	Satellite microscope scoring system
Microscope slide washing device	Automated chromosome aberration scoring system

3.0 APPLICATION OF MOLECULAR BIOMARKERS

Analyzing molecular biomarker responses to radiation exposure is a novel approach that can complement conventional chromosome-aberration assays and may significantly enhance biological dose assessments. Still in its infancy as a scientific discipline, radiation-responsive molecular biomarkers include proteins, gene expression, and DNA mutations. At AFRRI, we initiated studies to identify, evaluate, and validate molecular biomarkers that may provide diagnostic information for acute and prior radiation exposures. We are also exploring high-capacity, real-time detection technologies for nucleic acids or protein biomarkers. This technology would be useful in radiation accidents for forward-field dose assessment.

3.1 Radiation-Responsive Molecular Biomarkers

Recent technological advances in genomics and proteomics have contributed to the discovery of a plethora of radiation-responsive biomarkers. A few highly overexpressing sentinel radiation-responsive targets have been identified from an array of distinct gene expression profile responses [Amundson 2001]. Hofmann and colleagues [1990] reported radiation-induced increases of serum amylase in 41 patients, following either whole-body irradiation or irradiation of the head and neck regions. Becciolini and colleagues [2001] recently advocated the use of biochemical (e.g., serum amylase and tissue polypeptide antigen) dosimetry for prolonged spaceflights.

At AFRRI, using an *in vitro* model system of HPBL, we identified a candidate nucleic acid biomarker (i.e., gene expression target) responsive to ionizing radiation. A dose-dependent elevation in Ha*ras* gene expression levels was demonstrated using Northern blot analysis 17 h after exposure to 250-kVp x rays (25 to 100 cGy; 1 Gy/min) [Blakely 2002a, Miller 2002]. We also studied the effect of interindividual variation in Ha*ras* expression in healthy human donors. HPBL were given *in vitro* to 0- and 75-cGy ^{60}Co gamma rays (25 cGy/min dose rate) and incubated at 37 °C for 17 h after irradiation. Among the 11 donors, the control levels of Ha*ras* expression, relative to β–actin levels, were consistently low relative to a nearly uniform ≅ six-fold increase after a 75-cGy dose [Blakely 2002b]. Similar proto-oncogene studies, using a murine model system [Blakely 2003a, Miller 2002], demonstrated radiation-responsive gene expression targets after *in vivo* exposure.

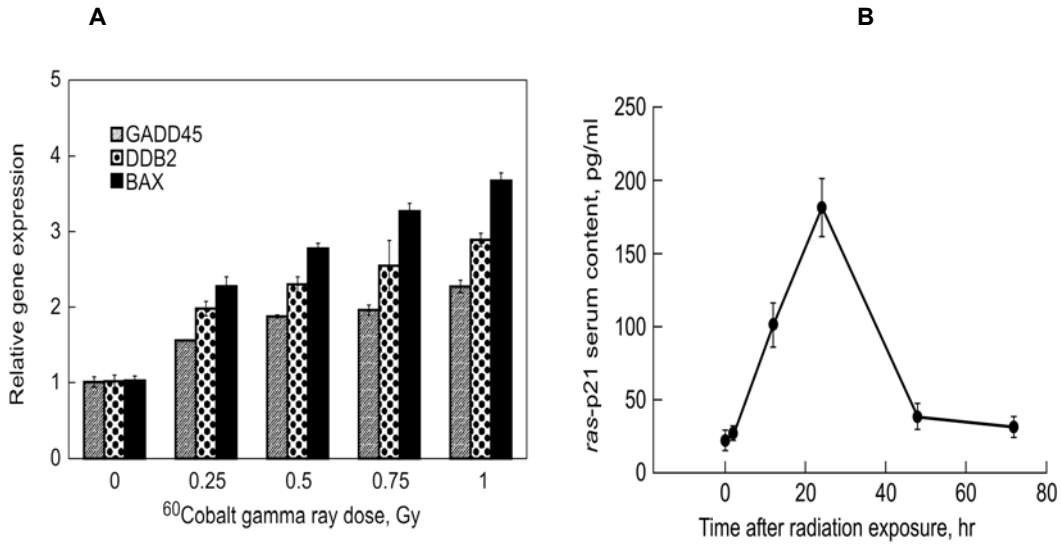
Use of a multiplex fluorogenic PCR analysis system is likely to improve the diagnostic utility for radiation dose assessment because the results are obtained rapidly. Protocols for detecting radiation-responsive gene expression, proto-oncogene Ha*ras* [Blakely 2002a; 2002b], and DNA repair gene GADD45 [Grace 2002,

Grace 2003] targets are developed using the real-time polymerase chain reaction (RT-PCR) assay. Identification and quantification of radiation-specific gene expression requires the measurement of multiple gene-expression targets. This will also increase sensitivity. Toward this goal, primers and Taqman probes are developed to evaluate additional candidate radiation-responsive targets (GADD45, DDB2, BAX). Figure 2, chart A shows the results of radiation response 24 h after 0 to 1 Gy [Blakely 2003a, Grace 2002].

An *in vivo* irradiation murine model system was further used to characterize candidate radiation-responsive *ras*-p21 proto-oncogene protein changes in blood. Similar to our *in vitro* data [Blakely 2003b], 25-cGy gamma rays resulted in detectable serum levels of *ras*-p21. An initial progressive increase and peak value of *ras*-p21 protein were observed at 24 h after irradiation, followed by a decline to control values at 48 and 72 h (Figure 2, chart B).

While nucleic acid and protein biomarkers appear to be of potential diagnostic utility, significant additional validation and assay optimization research are required. Still to be investigated are the effects of confounding factors such as the influence of radiation quality, the persistence of the endpoints after exposure, hyperthermia, and dose assessment methods in partial-body exposures.

Figure 2. Illustration of the responses of various biological indicators following exposure to ionizing radiation. Panel A. Dose-responses of multiple gene expression targets (GADD45, DDB2, and BAX) by multiplex real-time RT-PCR assay. The x-axis illustrates the reported nominal doses ranging from 0 to 1 Gy (dose rate, 0.1 Gy/min). The data were obtained at 24 h after irradiation [Blakely 2003a, Grace 2002]. Panel B. Radiation–responsive changes in the expression of *ras*-p21 in blood serum of 25 cGy irradiated rodents [Blakely 2003a].



3.2 Analytical Platforms for Measurement of Molecular Biomarkers

The ability to assess individual radiation exposures in a forward field will immensely help military operational and medical units. Recent rapid advances in nucleic acid biomarker analysis systems have made forward-field individual dose assessment possible. For example, new gene chip technology makes it possible to rapidly

monitor changes in both gene and protein expression. Similarly, real-time rapid fluorogenic PCR assay systems are commercially available as research and clinical laboratory systems. Military operations rely on molecular biology analysis platforms like those in reference laboratories for "reach-back" evaluations (e.g., samples transported from a forward field to a clinical, service, or research laboratory).

At AFRRI, we have initiated studies to optimize protocols and analytical systems for rapid measurement of radiation-responsive molecular biomarkers. A quadruplex and quantitative reverse transcriptase – PCR assay was developed using a 96-well, closed-plate format suitable for extracted RNA from whole blood [Grace 2003]. The simultaneous measurement of four amplicons in a single reaction using a closed-plate format provides significant cost and labor savings. Progress has also been made to rapidly detect blood serum protein biomarkers using a microsphere multi-analyte assay system, Luminex™-100 [Muderhwa 2003]. This technology is based on microscopic spherical polystyrol particles that serve as a solid phase for molecular detection reactions measured using a flow cytometer equipped with a 96-well microtiter plate platform. The system allows simultaneous, multiple detection reactions in very small sample volumes.

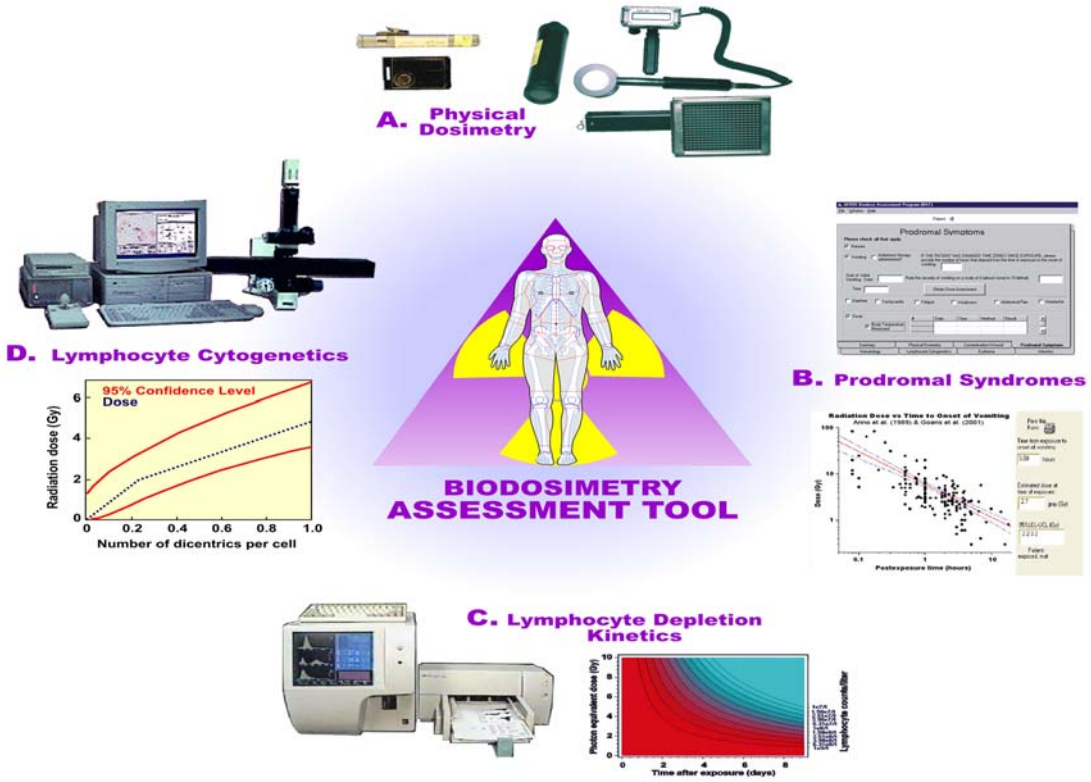
Deployable configurations of fluorogenic PCR assay systems have been used in field military operation units for pathogen detection [Belgrader 1998]. Significant efforts are under way to further miniaturize diagnostic equipment for nucleic acid sequence and antigen-based biosensor detection technologies [Blakely 2003b]. Dual-use applications of these and other diagnostic systems for radiation exposure assessment are essential to conserve the footprint required to equip forward deployable military laboratories and first responders.

4.0 BIODOSIMETRY ASSESSMENT TOOL SOFTWARE AND HEMATOLOGY

A U.S. Army-specific military requirement defines the need for a postexposure biodosimetry assessment tool to facilitate triage in the field, specifying that data collected should be in digital form for efficient transfer and availability to medical planners on the operational unit staff. The proposed STANAG 2474, NBC/MED of NATO, "***Determination and Recording of Ionizing Radiation Exposure for Medical Purposes,***" discusses the requirement for determining and recording of ionizing radiation exposure for medical purposes. The AFRRI Biological Dosimetry Team developed and released on the AFRRI website (www.afrri.usuhs.mil) the radiation casualty management software application BAT. This software is also suitable for civilian use [Sine 2001]. BAT is designed (a) to promote rapid collection of data for early use following a radiation exposure incident, (b) to provide diagnostic information and therapeutic guidance to manage radiation casualties, (c) to record related clinical information (e.g., extent of contamination, wounds, infection) necessary for proper medical care of radiation casualties, and (d) to archive collected data for later use. BAT, designed primarily for use within hours to days after a radiation disaster or exposure of personnel in a radiation environment, equips healthcare providers with diagnostic information, obtained via various modules, as illustrated in Fig. 3, that are germane to the management of human radiation casualties.

The guidance for collecting this diagnostic information is obtained primarily from preliminary physical dosimetry (Fig. 3 A) at a disaster site or after the discovery of the presence of personnel in a radiation environment and is secondarily aided by prodromal signs and symptoms (Fig. 3 B), serial lymphocyte counts (Fig. 3 C), and triage lymphocyte cytogenetics (Fig. 3 D). Detecting the presence of a radiological environment via physical dosimetry, where a population is exposed, may require a multiparametric approach and the use of several standard technologies. Examples of these include radiation detection meters that provide both dose and dose-rate information, portable meters that detect the presence of radioactive contamination, and self-reading personnel dosimeters. BAT provides structured templates to record this physical dosimetry information useful in the medical management of radiation casualties and in helping to rapidly triage individuals.

Figure 3. Centrality of Biodosimetry Assessment Tool (BAT) for biological dose assessment in radiation disasters for casualty management.



Similarly, BAT allows the recording of prodromal signs, symptoms, and erythema (Fig. 3B). Typical human symptoms in reaction to prompt total-body ionizing irradiation in the dose range of 0.5 to 30 Gy have been described by Anno [1989]. Recording of these radiation-induced signs and symptoms (e.g., nausea, vomiting, headache, and fever) before and during the course of medical management of radiation casualties will help triage and guide available treatment options.

Exposure to radiation causes a predictable depletion in lymphocytes in a time- and dose-dependent manner (Fig. 3 C). Deployable small foot-print point-of-care blood cell counters allow the medical professional to obtain on-site serial lymphocyte numbers and to determine lymphocyte depletion kinetics. A preliminary estimate of radiation dose in the region between 1 and 10 Gy (photon equivalent) can be obtained by this method. The contour of lymphocyte depletion kinetics following photon radiation exposure in human beings is shown in Fig. 3 C. Normal lymphocyte numbers in healthy individuals ranges between 1500/mm^3 and 3500/mm^3. Radiation doses as low as 0.2 Gy cause lymphocyte death in interphase, resulting in a severe depletion in the absolute count. For example, 1200 to 1500 lymphocytes/mm^3 at 24 h after irradiation may indicate a potentially lethal dose [Goans 1997]. However, due to the transient nature of radiation-induced lymphocyte depletion, the usefulness of this biodosimeter is limited to a few days (< 10) postexposure. Serial lymphocyte counts can be recorded in BAT program to convert them into dose predictions, using a lymphocyte depletion kinetic model based on previous dose responses in radiation accidents [Goans 1997].

The usefulness of lymphocyte cytogenetics and laboratory automation is discussed in section 2.3. In the triage dose prediction model for the dicentric assay, which used a calibration curve for ^{60}Co gamma radiation, a predictable radiation dose along with 95% confidence intervals, obtained by analyzing 50 metaphase spreads, plotted as a function of varying numbers of dicentrics in an exposed individual's blood in Fig. 3 D. With this dose prediction model, one can triage a large number of potentially irradiated individuals for radiation doses. However, this definitive and diagnostic individual biodosimetry is performed in a "reach back" cytogenetic laboratory.

5.0 CONCLUSIONS

We are developing and validating simple, rapid, and automated biodosimetry solutions for radiation dose assessment for military and civilian applications. An integration of multifaceted physical, hematological, cytogenetic, and molecular biodosimetry solutions is quintessential to determining immediate health risks to military personnel as well as civilian populations after a radiation disaster or the discovery of the presence of personnel in a radiation environment. BAT is central to AFRRI's multifaceted, multiparametric, and integrated biodosimetry system that adequately and fully addresses the need for triage of radiation casualties and aids in effective clinical management of potentially or actually exposed individuals.

Toward those ends, AFRRI has addressed the following tasks.

- We have established a "reach-back" cytogenetic biodosimetry reference laboratory that uses conventional bioassays as well as dicentric and premature chromosome condensation assays for provide definitive analyses of biological samples.
- We are developing and validating forward-deployable, biological dosimetry assays for rapid radiation dose assessment, with an emphasis on the use of diagnostic platforms based on molecular biology.
- We are integrating the biodosimetry data in a suitable software platform (i.e., BAT) to assist in the medical management of radiation casualties.

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SYMPOSIA DISCUSSION - PAPER 24

Authors Name: Dr Prasanna (US)

Discussor's Name: Dr Rios-Tejada (SP)

Question:
Is there relevance of biodosimetry in air crews who fly high and at high latitudes?

Author's Reply:
The application is evident and they are pursuing effort in that environment. (Relevant application to Commercial Aviation and Military Aviation in certain circumstances).

Real-Time Polymerase Chain Reaction Assays for Rickettsial Diseases

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ABSTRACT

Introduction/Rationale: *Arthropod-borne rickettsial diseases are found worldwide and have been the cause of significant amounts of suffering, disability and fatalities among both military and civilian populations throughout history. Because of the similarity to many infectious diseases in signs and symptoms rickettsial diseases are difficult to diagnose clinically. Moreover, due to the time it takes for antibodies to develop and the low concentration of rickettsial agents in the blood stream the diseases are also difficult to diagnose by laboratory methods. For that reason we have developed real-time PCR assays to detect rickettsial disease agents both at the genus and the species level.*

Methods: *Real-time PCR assays were developed to identify: 1) pathogenic* ***Rickettsia****; 2)* ***Rickettsia prowazekii*** *and* ***R. rickettsii****, the etiological agents for epidemic typhus and Rocky Mountain spotted fever (RMSF) and potential BW agents;* ***R. typhi*** *and* ***R. felis*** *the flea-borne typhus disease agents and* ***Orientia*** *(formerly* ***Rickettsia) tsutsugamushi*** *the scrub typhus agent. The assays utilize molecular beacon probes, which fluoresce when they encounter the target DNA sequence. By manipulating the annealing temperature, and magnesium, probe and primer concentrations of the assays, the optimal conditions were determined. A panel of 22 strains of rickettsiae, 20 strains of orientiae and 19 species of non-rickettsial agents were used to determine the specificity of the assays. Plasmids encoding the target sequences were used to calculate the sensitivity of the assays.*

Results: *The rickettsial real-time assays were found to be specific:* ***Rickettsia*** *assay only was positive for rickettsial and not other bacterial nucleic acid; R. prowazekii assay detected four strains of* ***R. prowazekii****, but not* ***R. typhi****, any of eight spotted fever rickettsiae,* ***O. tsutsugamushi*** *or 11 non-rickettsial bacteria.* ***R. rickettsii*** *assay detected two strains of R. rickettsii but not any other spotted fever or typhus rickettsiae.* ***R. typhi*** *and* ***R. felis*** *only reacted to the DNA extracted from* ***R. typhi*** *and* ***R. felis.*** ***O. tsutsugamushi*** *assay detected 20 strains of* ***Orientia*** *but did not react to 17 strains of* ***Rickettsia*** *or 19 species of unrelated bacteria. The sensitivity of the assays was quite good with detection routinely at the level of 3 to 10 copies per reaction.*

Conclusion: *These real-time PCR assays were found to be capable of detecting rickettsial diseases agents quickly and with great sensitivity and specificity.*

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

1.0 INTRODUCTION

Arthropod-borne rickettsial diseases are found worldwide and have been the cause of significant amounts of suffering, disability and fatalities among both military and civilian populations throughout history. Because of the similarity to many infectious diseases in signs and symptoms rickettsial diseases are difficult to diagnose clinically. Moreover, due to the time it takes for antibodies to develop and the low concentration of rickettsial agents in the blood stream the diseases are also difficult to diagnose by laboratory methods. For that reason we have developed the very sensitive and specific real-time PCR assays to detect rickettsial disease agents.

1.1 Rickettsial Diseases

Rickettsial diseases are caused by infection with obligate intracellular Gram negative bacteria transmitted by arthropod vectors (see Table I). Because of their obligate host cell requirement these rickettsial agents may not be grown up on artificial media, but must be grown in living cells such as those found in tissue culture, fertilized eggs or laboratory animals. Rickettsial diseases are similar in that they commonly produce high fever and severe headache in patients. Rash may be seen, especially in typhus, some of the spotted fever diseases and scrub typhus. Because the signs and symptoms of rickettsial diseases are not very distinctive they are often confused with many other infectious diseases (e.g. malaria, dengue and typhoid fever). The pathology of rickettsial diseases is associated with the infection of endothelial cells by the rickettsial agents, producing disease in potentially all organs and tissues of the body. The rickettsial diseases are normally self-limiting though they may be quite severe and life threatening illnesses. Antibiotics, such as one of the tetracyclines and chloramphenicol generally produce dramatic recuperative effects seen within 24-48 hours. However, antibiotic resistance has recently been reported, so new treatment modalities and/or vaccines need to be discovered (Strickman 1995, Watt; Corwin 1999).

1.2 Military importance of Rickettsial Diseases:

Rickettsial diseases have played havoc on military operations throughout history (Rapmund, Kelly DJ 2002). The particular diseases and some of the outcomes of rickettsial diseases on military operations are given below.

1.2.1 Epidemic typhus

Epidemic typhus is believed to be the “plague” that devastated Athens during the Peloponnesian War, and decimated Napoleon’s “Grande Armee” of 500,000 men that crossed the Nieman River into Poland to attack Russia in 1812. The Grande Armee returned to Poland in December with only 40,000 men. In addition to devastating military units, epidemic typhus commonly inflicts high morbidity and mortality upon poor, displaced and institutionalized populations (Raoult 1997, Raoult 1998, Tarasevich). During 1918-22 in Russia it is estimated that 2-3 million people (military and civilians during WWI and the Russia civil wars) died of typhus (Patterson). More recently (1997) in Burundi 45,558 typhus cases were diagnosed among the inhabitants during their country’s civil war (Raoult 1998). Because of epidemic typhus debilitating effects on military and civilian populations, it has been described as a potential BW agent (Eitzen). Moreover, due to its stability in dried feces of human body lice, its infection of humans by inhalation of aerosols, and the fact that just one organism can cause disease, *R. prowazekii* is considered a potential biological threat to be used by terrorists and rogue countries. This is more a concern now with the ability to produce in the laboratory antibiotic resistant strains, for which there is currently no FDA approved vaccine available to protect against (Kelly 2002).

1.2.2 Murine typhus

Murine (endemic) typhus is commonly found throughout the world (Azad). However, it was not until the early 1900’s that murine typhus was determined to be a separate disease from epidemic typhus and therefore its impact on military operations was unknown through WWII. Since then, murine typhus has been found to

have been a major cause of fevers of unknown origin (FUO) during the Vietnam War, especially among US soldiers stationed in bases and cantonments (Azad). Because of its worldwide distribution, murine typhus is a potential threat to military deployments everywhere (Miller, Corwin 1997, Raoult 1998).

1.2.3 Spotted fever

The spotted fevers group (SFG) include the agents that cause Rocky Mountain spotted fever (RMSF), Mediterranean spotted fever, African tick bite fever, Queensland tick typhus, and 10 other pathogenic and 20 other non-pathogenic rickettsiae. SFG rickettsiae have not caused major epidemics among military personnel similar to those associated with epidemic typhus. However in recent years, they have caused some significant outbreaks among U.S. military units training in CONUS and OCONUS and in one soldier returning from Somalia (Williams). During 1989, a military unit that trained in Arkansas and Virginia contracted RMSF. The seropositivity rate for those members that went only to Arkansas was 38% and for those that went just to Virginia was 13%. Forty-four percent of these individuals received medical treatment for their illnesses. In 1990, during exercises in Fort Chaffee, Arkansas, 30 individuals (n=1194) seroconverted to RMSF (Yevich). During a field-training mission in Botswana (1992), approximately 30% of 169 US soldiers sought medical attention due to an outbreak of African tick bite fever (Smoak). These outbreaks, the high exposure of military personnel to highly endemic areas and the rising incidence of RMSF at home (Treadwell) suggest that spotted fever diseases are a threat to military personnel training in tick-infested areas (Goddard).

1.2.4 Scrub typhus

Scrub typhus is caused by infection with *Orientia* (formerly *Rickettsia*) *tsutsugamushi*. It occurs in Asia, northern Australia and the western Pacific region, an area in which approximately one million cases occur each year and over one billion people are at risk for contracting the disease. During WWII, scrub typhus was second only to malaria as a leading cause of lost man-days in the Pacific Theater and in some locations feared more than malaria because of a high mortality rate that could not be controlled (Rapmund). During the Vietnam War scrub typhus was a leading cause of FUO, especially among those individuals deployed to jungle/rural areas (Reiley). Most recently scrub typhus has affected US military personnel conducting JTF-FA missions in Indochina, where possible drug resistance exist (Corwin 1999) and among US Marines training at Camp Fuji, Japan (Jiang 2003a). Moreover, drug resistant scrub typhus reported in Northern Thailand (Strickman, Watt) has increased the need to develop an effective vaccine (Kelly 2002).

1.3 Diagnoses of Rickettsial Diseases

Because of the similarity to many infectious diseases in signs and symptoms rickettsial diseases are difficult to diagnose clinically. Therefore, physicians often rely on laboratory tests to aid them in diagnosing rickettsial diseases. However, due to the time it takes for antibodies to develop and the low concentration of rickettsial agents in the blood stream the diseases are also difficult to diagnose by laboratory methods. Some of the current methodologies used in the laboratory include:

1.3.1 Serologic Assays

Serological assays including indirect immunofluorescent assay (IFA), enzyme-linked immunosorbent assays (ELISA) and Western blot analysis that are currently performed for epidemic typhus, murine typhus, spotted fever and scrub typhus using whole cell antigen preparations produced in our biological safety level (BSL)-3 laboraotories (Dasch, Halle, Raoult 1989, Richards 1993). Recent development of ELISA and Western blot assays for scrub typhus have been completed utilizing recombinant proteins (e.g. Kp r56, a recombinant protein representing a truncated 56 kDa outer membrane immunodominant antigen from the Karp strain of *O. tsutsugamushi*) (Ching 1998, Ching 2001, Jiang 2003a). The benefits of using recombinant proteins in these assays are the ease and safety in producing the proteins which are very stable as opposed to the whole cell antigen preparation that requires a BSL-3 laboratory, and is very labor intensive and time consuming to produce (Ching 1998).

1.3.2 Molecular biological Assays

Because rickettsial diseases are often misdiagnosed and detectable antibody levels are not seen until after day ten of the illness (Raoult et al 1986) a more rapid means of diagnosis is desired. Nucleic acid based antigen detection assays have been used to successfully identify rickettsiae in clinical, epidemiological, entomological and research specimens. The polymerase chain reaction (PCR) based assays have been used to detect the nucleic acid of rickettsiae responsible for typhus, spotted fever and scrub typhus (Tzianabos, Webb, Carl, Furuya, Kelly 1994).

1.4 Recent Development of Real-time PCR Assays for Rickettsial Diseases

Very recently, a more specific, sensitive, quantitative and faster nucleic acid based antigen detection assay has been developed to detect infectious disease agent nucleic acid. This assay, quantitative real-time PCR (qPCR), has been utilized in our laboratory to develop rickettsial agent specific detection assays. The development, sensitivity and specificity of these assays are described in this presentation.

2. Materials and Methods

The quantitative real-time PCR assays were performed utilizing a master mix prepared containing ddH_2O, pre-mixed reagents OmniMix HS (Cepheid, Sunnyvale, CA), forward and reverse primers, molecular beacon probes, and $MgCl_2$ (Invitrogen, Carlsbad, CA). Each 25 ml reaction contained: 25 mM of HEPES buffer, 0.2 mM of dNTPs, 5 mM of $MgCl_2$, 1 ul of template DNA, 1.5 units of Takara Taq DNA polymerase; and 0.3, 0.2, 0.3, 0.5, 0.4, 0.1 and 0.5 uM of forward and reverse primers, and 0.4, 0.3, 0.2, 0.5, 0.4 and 0.2 uM of probe for the specific qPCR , rickettsiae 17 kDa, *R. prowazekii ompB*, *R. typhi ompB, R. felis ompB, R. rickettsii ompB, O. tsutusgamushi* 47 kDa, bacterial 16S rRNA, respectively. The qPCR were performed in a SmartCycler thermocycler (Cepheid, Sunnyvale, CA). The temperatures and cycle parameters included: initial denaturation of 3 minutes at 94^{o}C; and 50 cycles of denaturation (94^{o}C for 5 sec) and annealing/elongation (60^{o}C for 30 sec). CT (crossing the threshold) value, a positive reaction, was identified by the experimental samples producing fluorescence greater than the calculated threshold value based upon background fluorescence measured during amplification or an adjusted value based on its fluorescence strength. "No template controls" produced at the same time and under the same conditions as the experimental positive control samples were consistently negative (did not cross the threshold of background fluorescence).
To determine the sensitivity of the real-time PCR assays, serial dilutions of known concentrations of the target sequence in plasmids for each assay, were assessed as previously described (Jiang 2003b, Jiang 2004).

3. Results

3.1 Epidemic typhus qPCR

Identification of *R. prowazekii* by qPCR was accomplished to the level of detection of three copies of target sequence per microliter. In addition, the assay detected all four strains of *R. prowazekii* evaluated (Breinl, Madrid E, Ananiev and Cairo). However, it did not give a false signal for two strains of the closely related *R. typhi* (Wilmington and Museibov), or any of 8 spotted fever rickettsiae or *O. tsutsugamushi*. In addition, the assay did not falsely detect DNA from 11 unrelated bacteria or from host cell material used to propagate certain bacteria (Table II). Thus, this assay is very sensitive (3 copies/ ul) and specific.

3.2 Murine typhus and cat-flea typhus qPCR

Identification *R. typhi* and *R. felis*, members of the typhus group and spotted fever group rickettsiae, respectively, cause disease in humans following the bite of an infected flea. Because these agents have the capability of being transmitted simultaneously, and produce similar diseases, we developed a qPCR assay for each agent based upon different target sequences of the *ompB* gene. Both assays are agent specific and very sensitive. Utilizing the panels of rickettsial and non-rickettsial bacterial DNA panels, each agent (two strains for *R. typhi* Wilmington and Museibov) and a single isolate of *R. felis* were consistently detected by their corresponding assays, but these same assays did not falsely detect the other agent, other rickettsial species or non-rickettsial species nucleic acid preps (Table II). The assays were sensitive enough to detect as low as three copies of the target sequence in 1 ul samples.

3.3 Rocky Mountain spotted fever qPCR

R. rickettsii, the etiologic agent of RMSF was detected utilizing a qPCR assay based upon an agent specific partial sequence of the *ompB* gene. The assay was sensitive enough to detect down to three target sequence copies per microliter. In addition, the assay was specific enough not to detect two strains of *R. rickettsii* R and 364-D, but did not falsely react with nucleic acid from any of the other 8 spotted fever group or the typhus group rickettsiae. It also did not react falsely with *O. tsutsugamushi* or 11 non-rickettsial nucleic acid preparations.

3.4 Scrub typhus qPCR

The detection of *O. tsutsugamushi* by the scrub typhus qPCR assay was able to detect 20 different strains of *O. tsutsugamushi*, but did not produce false positive responses to 17 rickettsial or 19 bacterial nucleic acid preparations. These preparations were evaluated by other nucleic acid detetion assays (17 kDa, 16S rRNA genes) to ensure that the negative reactions were due to specificity of the qPCR assay and not because there was a lack of appropriate nucleic acid in the preparations (Table II). The sensitivity for this assay like the others was efficient enough to detect down to three copies of the target sequence.

4.0 Discussion

With the development of the polymerase chain reaction (PCR), where agent-specific nucleic acid can be amplified a million fold, rickettsial DNA can be readily detected with great sensitivity in clinical specimens such as blood and tissue samples as well as in arthropod vectors (Tzianabos, Webb, Carl, Furuya, Kelly 1994, Richards 1997, Williams). This sensitivity has been enhanced by the development of the nested PCR assays, which are performed using two separate amplification steps. In addition to detection, the amplicon can be used for restriction fragment length polymorphism typing or sequence analysis. Quantitative real-time PCR assays are as sensitive as nested PCR assays but have the additional advantages of increase specificity, faster results and quantitative information, useful for clinical monitoring of appropriate response to treatment and prognosis, and for experimental studies.

In this report we describe the development of six qPCR procedures with agent specific fluorescent probes able to detect pathogenic rickettsial agents. These assays were developed initially as standard PCR (sPCR) assays, albeit with resultant amplicons of only about 100 bp. The assays were optimized utilizing DNA extracted from the target organism (e.g. *R. prowazekii* Breinl, *O. tsutsugamushi* Karp, etc) and following the selection of an appropriate probe they were further optimised for use in the real-time PCR assay. The specificity of the assays was subsequently evaluated utilizing a panel of closely and distantly related bacteria. The panels included nucleic acid preparations from 17 strains of *Rickettsia,* 20 strains of *O. tsutsugamushi*, and 19 species of other bacteria (Table II). Production of the panels was described previously (Jiang 2004).

Each of the six assays evaluated showed the ability to detect specific target selection, with all strains of the target entity producing positive reactions and no positive responses from the unrelated *Rickettsia*, other bacteria nucleic acid samples assessed, or the host cell DNAs in which the obligate intracellular bacteria were cultivated, indicating that the qPCR assays were specific for their target agent. The integrity of the *Rickettsia* and bacterial DNAs were confirmed with a qPCR assay based upon the conserved *Rickettsia* 17 kDa antigen gene and a sPCR assay for the 16S rRNA gene (Jiang 2004).

To determine the sensitivity of the qPCR assays we utilized plasmids containing the target sequences. These included fragement A of the open reading frame of the *ompB* of *R. prowazekii* and *R. typhi,* and the open reading frame sequence of the Kato 47 kDa gene that was ligated into the plasmid VR1012 (Vical) (pWMC-Kt47). The other qPCR assays utilized the amplicons produced by the sPCR to ligate into the Topo plasmid according to the manufacturer's instructions. The plasmids' concentrations were determined by OD reading at 260 nm. Serial ten-fold dilutions of the plasmids in molecular biology grade water were performed resulting in final target concentrations of 10^7 to 10^0 copies/µL. The assays consistently detected between 3-10 copies of the target sequence per reaction.

In summary, this paper describes six qPCR assays for detecting rickettsial pathogens that are sensitive (3-10 copies/reaction) and specific. These assays are currently under evaluation for effectiveness in detecting target sequences within arthropod vectors, blood, urine and other background matrices.

5.0 Acknowledgements

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Table I: Rickettsial Diseases, Rickettsial Agents and their Arthopod Vectors

Group	Disease	Agents	Arthropod Vector
Typhus	Epidemic Typhus	*Rickettsia prowazekii*	Human Body Louse *(Pediculus humanus)*
	Murine Typhus	*Rickettsia typhi*	Oriental Rat Flea *(Xenopsylla cheopis)* & Cat Flea (*Ctenocephalides felis)*
Spotted Fever	Rocky Mountain Spotted Fever	*Rickettsia rickettsii*	Tick *(Dermacentor variabilis)*
	Mediterranean Spotted Fever	*Rickettsia conorii*	Tick *(Rhipicephalus sanguineus)*
	African Tick Bite Fever	*Rickettsia africae*	Tick *(Amblyomma* sp*)*
	Queensland Tick Typhus	*R. australis*	Tick *(Ixodes holocyclus)*
	Cat Flea Typhus	*Rickettsia felis*	Cat Flea (*Ctenocephalides felis)*
	Rickettsialpox	*Rickettsia akari*	Mouse Mite *(Liponyssoides sanhuineus)*
Scrub Typhus	Scrub Typhus	*Orientia tsutsugamushi*	Chigger (Leptotrombidium)

Table II: Rickettsial specific qPCR with panels of rickettsiae and non-rickettsial bacteria nucleic acid preparations

Rickettsia Isolates	17 kDa sPCR	*R. prowazekii ompB* qPCR	*R. typhi ompB* qPCR	*R. felis ompB* qPCR	*R. rickettsii ompB* qPCR	Orientia tsutsugamushi 47 kDa qPCR	16S rRNA sPCR
R. prowazekii Breinl	+	+	-	-	-	-	ND
R. prowazekii Madrid E	ND	+	ND	ND	ND	ND	ND
R. prowazekii Ananiev	ND	+	-	-	-	ND	ND
R. prowazekii Cairo	ND	+	ND	ND	ND	ND	ND
R. typhi Wilmington	+	ND	+	-	ND	-	ND
R. typhi Museibov	+	-	+	-	-	-	ND
R. bellii G2D	+	ND	ND	ND	ND	-	ND
R. canada MCK-29	ND	ND	-	-	-	ND	ND
R. rickettsii R(2D)	+	ND	ND	ND	+	ND	ND
R. sp. 364-D	+	-	-	-	+	-	ND
R. conorii ITT	+	-	-	-	-	-	ND
R. montana OSU 85-930	+	-	-	-	-	-	ND
R.africae EthSFC84360	+	ND	ND	ND	ND	-	ND
R. sharonii ISTT CW	+	ND			ND	-	ND
R. parkeri Maculatum C (CWPP)	+	-	-	-	-	-	ND
R. slovaca D	+	-	-	-	-	-	ND
R. japonica NT	+	-	-	-	-	-	ND
R. sibirica 3358	+	-	ND	ND	-	-	ND
R. rhipicephali CWPP	+	ND	ND	ND	ND	-	ND
R. honei TT118	+	ND	ND	ND	ND	-	ND
R. akari Str #29	+	-	-	-	-	-	ND
R. felis	+	ND	-	+	-	-	ND

Orientia tsutsugamushi Strains	17 kDa sPCR	*R. prowazekii* *ompB* qPCR	*R. typhi* *ompB* qPCR	*R. felis* *ompB* qPCR	*R. rickettsii* *ompB* qPCR	*Orientia tsutsugamushi* 47 kDa qPCR	16S rRNA sPCR
Karp	-	-	-	-	-	+	ND
Kato						+	
Gilliam						+	
TA678 PP						+	
TA686 PP						+	
TA716 PP						+	
TA763 PP						+	
TH1813						+	
TH1814						+	
TH1817						+	
TH1818						+	
TH1819						+	
TH1823						+	
AFC3						+	
AFPL12						+	
AF245						+	
AF312						+	
AF316						+	
AF338						+	
MAK110						+	

Other Bacterial Species	17 kDa qPCR	*R. prowazekii ompB* qPCR	*R. typhi ompB* qPCR	*R. felis ompB* qPCR	*R. rickettsii ompB* qPCR	Orientia tsutsugamushi 47 kDa qPCR	16S rRNA sPCR
Ehrlichia chaffeensis	ND	ND	ND	ND	ND	-	+
Neorickettsia sennetsu	-	-	-	-	-	-	+
N. risticii	-	-	-	-	-	-	+
Bartonella quintana	-	-	-	-	-	-	+
B. vinsonii	-	-	-	-	-	-	+
Francisella persica	-	-	-	-	-	-	+
Legionella pneumophila	-	-	-	-	-	-	+
L. bozemanii	ND	ND	ND	ND	ND	-	+
L. micdadei	ND	ND	ND	ND	ND	-	+
Proteus mirabilis	-	-	-	-	-	-	+
Escherichia coli	-	-	-	-	-	-	+
Citrobacter freundii	ND	ND	ND	ND	ND	-	+
Shigella flexneri	ND	ND	ND	ND	ND	-	+
Pseudomonas aeruginosa	ND	ND	ND	ND	ND	-	+
Vibrio cholerae	ND	ND	ND	ND	ND	-	+
Aeromonas hydrophila	ND	ND	ND	ND	ND	-	+
Staphylococcus aureus	-	-	-	-	-	-	+
Corynebacterium sp.	-	-	-	-	-	-	+
Salmonella enterica	-	-	-	-	-	-	+

Surveillance for Respiratory Infections in U.S. Military Populations Using Classic and Novel Diagnostic Techniques

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ABSTRACT

Military populations are historically susceptible to outbreaks of acute respiratory disease. These epidemics disrupt training schedules, place a heavy burden on the military medical system, cause significant economic losses, and ultimately impact troop readiness and mission accomplishment. The U.S. Naval Health Research Center (NHRC) has provided population-based viral respiratory surveillance in select US military populations since 1996. Although classical methods of diagnosis (culture) are the gold standard, these techniques are laborious and time-consuming. Novel diagnostic techniques were recently explored, and show promise for providing rapid results for large numbers of specimens.

1.0 NAVAL HEALTH RESEARCH CENTER RESPIRATORY DISEASE LABORATORY

Established in 1996, the Naval Health Research Center Respiratory Disease Laboratory was created to address concerns of morbidity caused by emerging and re-emerging respiratory pathogens within the Department of Defense (DoD) [1]. Critical at this time was the pending loss of the adenoviral vaccine that was in use in our recruits since the early 1970s. The vaccine's sole manufacturer was discontinuing production. Supplies of vaccine were rationed seasonally at recruit training centers until early 1999, when all supplies were exhausted. There was need to document the effect of this vaccine loss, thus initiation of surveillance efforts.

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The cornerstone of the developed program was population-based surveillance with laboratory diagnostic support at 8 recruit training sites within the Navy, Air Force, Army, Marines and Coast Guard (Figure 1). Dedicated civilian research associates (RA) were hired to perform the surveillance at each site, ensuring long-term success and minimizing the workload on local staff. The local RAs are responsible for monitoring sick call clinics and logs, and determining total number of individuals at each recruit training site that meet the case definition of: Oral temperature of 100.5°F, cough and or sore throat, and all non-bacterial pneumonias. From a selection of these individuals, a throat swab clinical specimen is taken, and stored at ultra-low temperatures. At least once a month, these samples are forwarded to our reference laboratory in San Diego for classic virology cell culture. RAs also collect the denominator data (the total number of recruits on site vulnerable to infection) at their respective recruit-training site. With this collected numerator and denominator information, rates are followed from season to season and year to year.

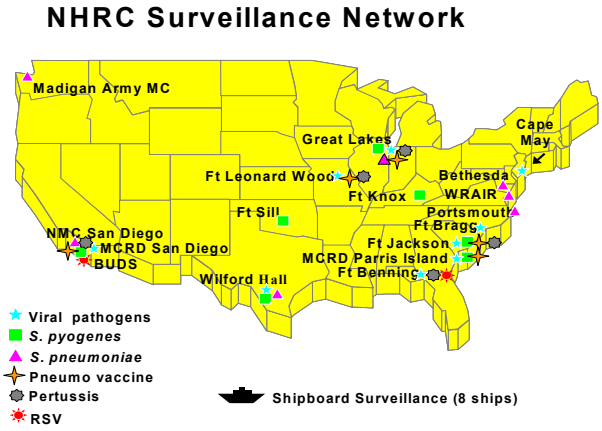

Figure 1

The data collected from this surveillance system since its inception in 1996 have been critical to the DoD. Numerous outbreaks caused by adenovirus have been documented with this laboratory-based surveillance system [2]. Critical data regarding the resurgence of adenoviral febrile respiratory illness in our recruits after the adenovirus vaccine was no longer available was also gathered [3, 4]. These data were invaluable for providing evidence that the adenovirus vaccine within our U.S. recruit populations was indeed needed; renewed efforts to find a new vaccine manufacturer were therefore initiated. Currently, Barr Pharmaceuticals is working to re-establish this vaccine. The currently projected availability year is 2009.

Figure 2 contains an example of information that is currently available on our internet site http://www.nhrc.navy.mil/geis/sites/nhrc.htm . This information is also shared via quarterly newsletters, and in weekly emails to concerned and engaged parties within the Department of Defense.

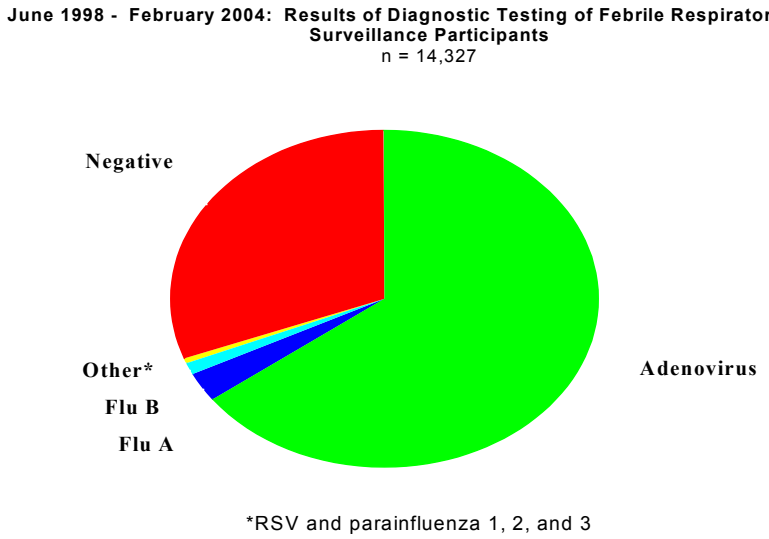

Figure 2: Results of diagnostic testing of samples received from recruits with a febrile respiratory illness. This diagnostic testing clearly shows adenovirus as the pathogen of greatest concern.

1.1 EXTENSION OF SURVEILLANCE TO REMOTE SETTINGS

As discussed, the developed febrile respiratory surveillance program proved very valuable within the U.S. recruit training settings. However, within the DoD, military members are exposed to a variety of other high-risk settings for acquiring respiratory infections. Morbidity from respiratory pathogens while on deployment can be crippling to mission accomplishment. To address these concerns, our surveillance capabilities were extended to a variety of other settings, often in remote sites.

Performance of surveillance onboard ships within the U.S. Naval Fleet is one such extension of our surveillance capabilities. As of March 2004, eight ships were taking part in our surveillance for febrile respiratory illnesses. Like surveillance in the recruit training setting, numerator and denominator data are collected, and a selection of individuals provide a diagnostic specimen for future testing. These samples are usually collected while the ship is at sea. They are stored at ultra low temperatures (liquid nitrogen or –70 degrees Celsius freezers), and upon return to their homeport at the end of their deployment, delivered to our respiratory disease laboratory for diagnostic workup. If a large respiratory outbreak were to occur at sea, appropriate supplies are on hand for collection of optimal diagnostic specimens at the time the outbreak is recognized. A special shipment could be arranged if needed for expedited laboratory processing. Figure 3 illustrates some of our results during the first 11 months of implementation in this setting.

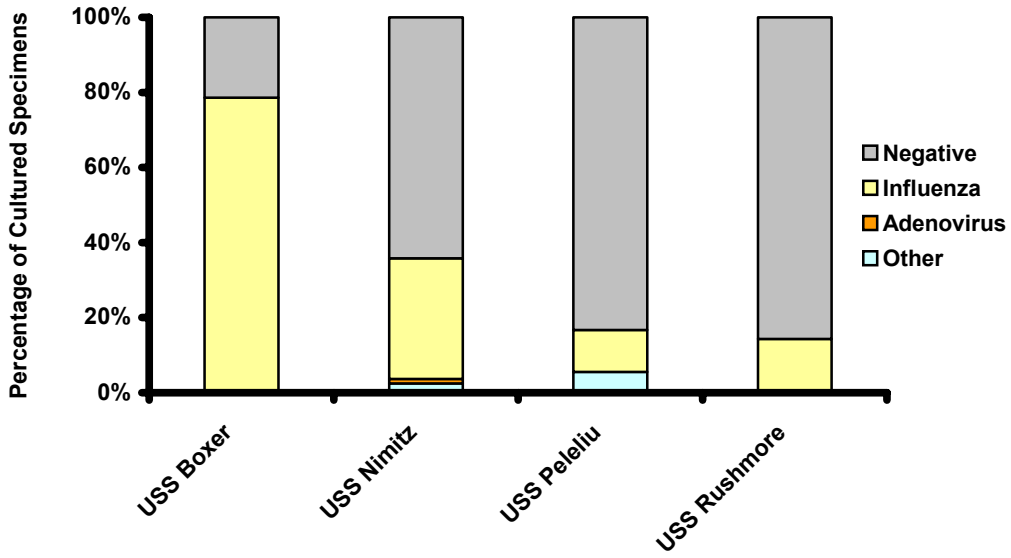

Figure 3: Results of diagnostic testing from samples acquired while on deployment from 4 U.S. Naval ships. Note influenza was diagnosed from samples on all 4 ships. Port stops prior to the outbreaks included Singapore and Hawaii.

Diagnostic testing capabilities were also provided to select ships that had Light Cyclers onboard. All consumables, controls, and an instruction manual were provided to each ship that requested diagnostic testing capabilities for influenza A, influenza B, and adenovirus. The testing takes approximately 4 hours for each pathogen. Although results were potentially available quickly, the testing still requires much time from the ship's laboratory technician. Given these time constraints, testing on-board ship was only rarely utilized.

Laboratory-based surveillance initiatives were also undertaken during the 2003 "Cobra Gold" exercise--a joint exercise located in Thailand. SARS transmission was not known to be present in Thailand in early 2003, and there were concerns that exercise participants could bring SARS into the country. Respiratory illness surveillance was initiated, including geographic exposure history for elucidating a SARS "suspect" case. Although no "suspect" SARS case was identified, 17 individuals met the case definition for febrile respiratory illness during this 4 week exercise; diagnostic specimens were obtained from 16. Laboratory testing identified influenza A in 7 samples (44%); sequence analysis on four of these demonstrated they were closely related to the Fujian-like influenza strain, which was the predominant strain found globally in 2003/2004. Two samples (13%) were positive for coronavirus OC43, 2 (13%) for respiratory syncytial virus, 1 (6%) for rhinovirus, and 4 (25%) were negative. Concern for SARS transmission was eased and knowledge of circulating respiratory pathogens was obtained as the logistics of implementing respiratory surveillance during a military training operation were overcome.

2.0 CLASSIC LABORATORY PROCESSING

Maintaining these classic methods of detection should always be high priority for any reference laboratory. They result in a critical product that none of the advanced molecular methods for pathogen detection are able to provide: a viable organism. The ability to *grow* the influenza virus is necessary for current influenza vaccine formulations. In addition, a viable organism allows testing of antigen/antibody interactions which can never be reproduced with only a portion of a non-viable genome. Complex tertiary and quarternary structures are absent in partial genome amplicons, so presence of the epitopes necessary to conduct such techniques is lacking. Our ability to perform in-vitro techniques that approximate the in-vivo antigen/antibody interaction is lost without viable, live organisms.

Clearly, classic methods of diagnosis for samples collected in our surveillance network have provided much-needed information. However, the potential disadvantages of laboratory-based surveillance as described are easy to discern. These classic methods are laborious, taking several weeks to process samples and requiring many man-hours for care of cell cultures. Consumables are numerous, from cells, to propagation media and reagents for performing detection methods such as immunoflourescence. There is also the need for specialized instrumentation such as the inverted microscope, the immunofluorescent microscope, and incubators. Results are not available in time for patient management decisions.

Another disadvantage of the classic methods is that any given cell line is only permissive to growth of a few viruses. Growing new, unknown pathogens would be unlikely in such a scenario. In addition, if a new virus was able to grow in the cells utilized, the detection methods require knowledge of what pathogen we are seeking. Antibodies can only be purchased against known organisms! Our ability to be sensitive to pathogen discovery is greatly reduced.

3.0 NOVEL DIAGNOSTIC TECHNIQUES

Molecular polymerase chain reaction (PCR) methods for detection are a frequently used alternative to classic cell culture methods. PCR offers the advantage of more timely results, often in 1 day or less. However, diagnosis is still essentially one specimen and one pathogen of interest at a time, and additional expensive instrumentation is required. Another disadvantage of these molecular techniques is that knowledge of the nucleotide sequence of the pathogen to be tested for is required. Pathogen discovery or identification of unknowns with these traditional PCR techniques is not possible. Multiplex platforms may allow detection of a few pathogens at a time, but often at the expense of sensitivity, with increasing risk of non-specific amplification or spurious band production ("primer dimers") as multiplexing efforts are increased. Newer "real-time" polymerase chain reaction such as the Light Cyler, Smart Cycler or TaqMan techniques make the need for gel electrophoresis for visualization of amplified product obsolete, thus reducing the potential turn-around time to hours. Again, additional costly instrumentation is required.

As we consider characteristics of the ideal diagnostic test, the following requirements should be considered:

1. Inexpensive for daily routine testing. Limited up front investments for instrumentation potentially could be supported.

2. Rapid, requiring hours rather than days or weeks.

3. Be useable in remote unsophisticated settings.

4. Not require extensive sample preparation prior to testing.

5. Be permissive to a variety of diagnostic specimens…from air samples to human samples to zoonotic samples.

6. Allow for processing large numbers of patient specimens for a large battery of pathogens simultaneously.

7. Be sensitive to unknown pathogens. The common pathogens, uncommon pathogens, and unknown pathogens should be detectable.

Clearly, these are challenging requirements to meet. In our new molecular age, however, the possibilities seem endless. A plethora of new sophisticated detection modalities are in various stages of development. One such technology is the micro-array format. Also requiring additional instrumentation, this technique does have the advantage of potentially testing a given specimen against a large battery of pathogens simultaneously. However, knowledge of the genetic sequence of the pathogen you are looking for is still needed with this technology.

One promising technology aggressively being pursued in our laboratory is called "Triangulation Identification for the Genetic Evaluation of Risks" (TIGER) through collaboration with Ibis Pharmaceuticals. This technology, although still in developmental stages, was successfully utilized in our surveillance efforts and fulfils many of the characteristics of the ideal testing platform.

The "triangulation" aspect of the TIGER acronym was coined from the concept of measuring MULTIPLE conserved and essential regions of pathogens present, as represented in Figure 4. There exist conserved regions of genomes that are essential for viability and replication of micro-organisms, and are therefore present in a genomically identical manner. Although the exact pathogens being sought may not be know, these conserved regions will be the same. Using PCR as the first amplification modality, these conserved regions are targeted and the region between is amplified. This nucleic acid sequence between the conserved portions differs in a pathogen specific manner.

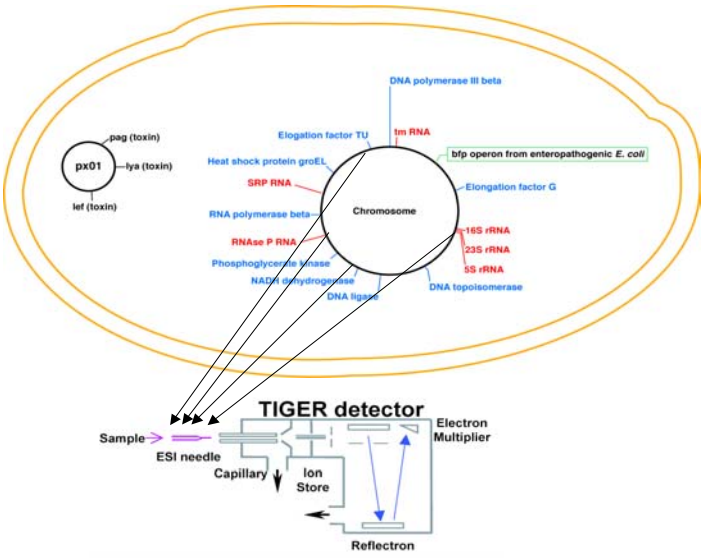

Figure 4: Triangulation identification of pathogens utilizing conserved genome portions for detection. In this manner, knowledge of the exact organism being targeted is not needed.

The TIGER technology, therefore, can take a complex mixture (specimen from any source: environmental, human, or animal), isolate the available DNA, and amplify from the conserved regions in this "triangulation" manner. A complex mixture of amplicons is produced with nucleic acid information between the conserved regions that is specific to their parent pathogen. These amplicons are then sprayed into a mass spectrometer that measures the specific molecular weight of each amplicon. Resolution of a complex mixture is achieved with high precision. The mass of each amplicon is determined to such high mass accuracy, that only one combination of nucleotides could result in that specific molecular weight. This specific nucleotide content is then often specific to only one pathogen, when results of all the triangulation amplicons are considered. See figure 5 and 6.

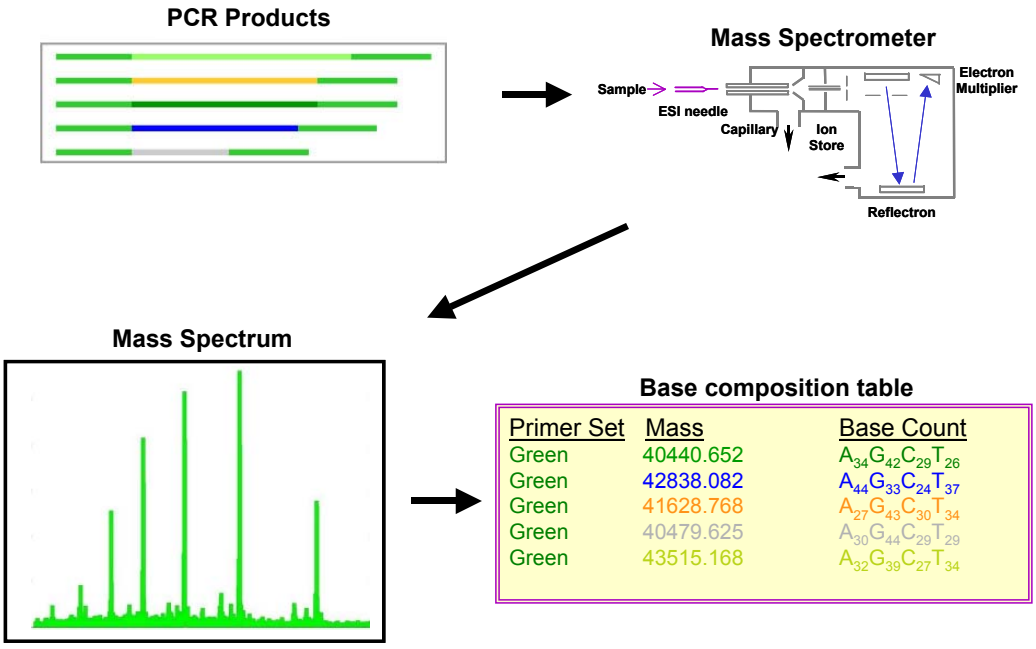

Figure 5: The process of determining the molecular weight of amplicons present in a complex mixture, and their specific base composition.

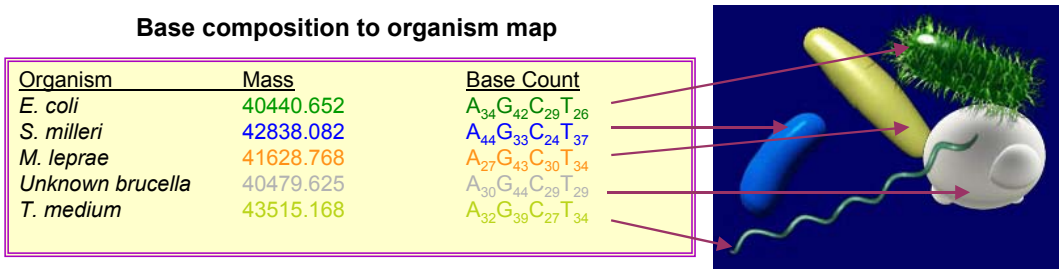

Figure 6: Taking the specific base composition and determining the pathogen of origin, using information from the triangulation and algorithms of known pathogen base count composition.

As a demonstration of the TIGER capabilities, an outbreak of Group A streptococcus (GAS, *Streptococcus pyogenes*) pneumonia among U.S. Marines was monitored and followed with this technique [5]. There was the need and desire to monitor the potential spread of the bacteria to other geographic sites. The questions that needed addressing: Are GAS outbreaks occurring in other geographic locations the same strain of GAS responsible for the Marine pneumonia outbreak? Is an unusually virulent clone causing these infections? Should we give prophylaxis to all groups that are exposed to troops from the original outbreak? Traditional classic and molecular methods of following the strains responsible for the illnesses were too laborious and time consuming to be effective epidemiologically. The TIGER technology was adapted for this purpose, and up to 600 samples could be processed overnight, giving sufficient resolution of genome variability or uniformity to follow the strains causing infection in other regions. We were able to quickly understand that the Marine GAS pneumonia strain was not causing wide spread illness in other military populations. Preventive medicine interventions were initiated with this information, information that was not attainable with traditional methods.

Adenovirus, as described above, is an important pathogen for military populations, particularly recruits. Knowledge of which serotypes are in circulation is important for renewed vaccination efforts. Traditional methods of determining serotype are again laborious and time-consuming. This TIGER technology was also successfully utilized for serotype determination of large numbers of adenovirus isolates.

Although additional instrumentation is needed with this TIGER technique, high throughput of original patient specimens against a countless number of potential pathogens is possible. In addition, using the broad priming techniques, amplicons that are not readily identifiable with given sequence information would alert one to the possibility of a unique pathogen being present. Many of the characteristics of the ideal diagnostic test could be fulfilled with this technology as it becomes refined.

4.0 DISCUSSION

Respiratory pathogens are of critical importance to the military. Only through laboratory-based surveillance efforts will we understand the pathogen specific risks in our different high-risk groups. Newer methods are being developed that will allow us to monitor and conduct this surveillance even more efficiently. We have a responsibility to remain knowledgeable about these developing techniques, as we strive to understand, diagnose and prevent illness among our constituents.

Classic techniques should not be abandoned, as their role is undeniably critical. However, for more timely capabilities at the point-of-care, alternative diagnostics must be pursued. Rapidly progressing technologies promise to provide greatly expanded capabilities in the near future, if we stay open to their potential. Validation of such techniques much be given priority, and must be thorough. As our ability to detect organisms becomes more robust, we must become more sophisticated in our ability to understand which organisms are actually responsible for disease. The commensal world must be better understood. However, if we remain dedicated to the cause, the rapid, accurate diagnosis of illness and heightened health and readiness status of those we are responsible for will be the reward.

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Psychological Support Pre-During and Post-Deployment

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ABSTRACT

From the early nineties until now over 35.000 servicemen were send abroad for their term. The Division of Ambulant Psychotherapy of the Royal Netherlands Land Army developed a comprehensive set of measures. A policy to secure maximum deployability and minimise the (long)lasting effects of stressful encounters during these operations. In this paper ten steps are presented which comprises this policy for psychological support pre- during and post deployment. One step of this policy, the after care questionnaire, will be presented in more detail.

Introduction

The psychological effects of war and peacekeeping upon soldiers are very well known. They comprise all problems from maladaptation after return, up to and including fullblown post traumatic stress disorders.

The Netherlands, although being a small country, has a large history of involvement in (UN) peacekeeping operations. It started with a peacekeeping operation in Albania in 1913, through Korea 1950, to Lebanon 1979-1985. In this last operation, UNIFIL, 8000 men and women from the Royal Netherlands Land Army (RNLA) served, of whom 9 were killed.

Some figures: Operations started in 1991 in Saudi Arabia and Iraq (with the Gulf war and later Provide Comfort), then Cambodia, Haiti, Angola, operations in 15 countries in all. From the RNLA about 20.000 served in the former Yugoslavia. At the moment about 1200 men and women serve in Bosnia, 650 in Afghanistan and about 200 in 10 other UN/NATO or EC-missions.

All together about 35.000 men and women, regular as well as –until 1996- conscript soldiers, were and are involved, since Lebanon until now.

Neither the UN, NATO nor the WEU have a doctrine or clear policy on psychological support before, during and after operations. Each participating country has it own responsibility in this matter.

The RNLA developed a comprehensive policy to secure maximum deployability and minimise the (long)lasting effects of stressful encounters during operations. This policy focuses upon the armed forces, before, during, and after missions of soldiers during peacekeeping operations, starting with initial psychological selection, up to and including veteran care, and thus set the stage in which all aspects of welfare during peace keeping operations fit very well. In more detail some results are given from the structural questionnaire surveys as an essential part of this policy.

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Two final remarks. There is no UN policy, nor a policy in NATO or WEU concerning psychological support. It is a national responsibility, and the remainder of this paper is based on the policy of the Royal Netherlands Land Army and the Royal Marechausee (or military police). The two other forces of the Netherlands; the Air Force and the Navy (including the Marines) implemented a similar policy, that differs in detail depending for example on the nature of the mission and their own health care system.

Secondly: Not only the NATO, the WEU, or the UN, but also the so called Non Governmental Organisations, like the Red Cross or Medicin sans Frontieres consider people in need, their responsibility. Apart from those two important organisations, the World Health Organisation knows over 450 non-governmental bodies with their own policies regarding psychological support.

Contributions of military psychology to UN-operations, principles.

A lot is learned from literature and personal contacts with colleagues from other countries; for example the experience of colleagues in Israel, the United States, especially after Vietnam, and Norway (the UNIFIL-study).

But of course there are our own experiences, with for example the veterans from the political conflicts in the former Dutch Indies 50 years ago, but still a current topic. Then Korea, and later Lebanon (1979-1985) where we had over 8000 men. After that operation three major studies into the problems of these UNIFIL-veterans (1980, 1987, 1989) were conducted. To give only one of the research results: in 1987 about 10 percent of our Lebanon-veterans still suffered from problems related to their mission: from maladaptation to PTSD. In those days however, hardly support was given by behavioural scientists or psychotherapists, mainly because there were only a few of them at that moment, and psychological support was not so well accepted as it is nowadays.

Research and the experiences of therapists have shown that soldiers who have problems coping with their experiences resulting from a mission, frequently withdraw from social contacts, feel themselves misunderstood and often deny that they have any psychological problem. These symptoms mean that military personnel with psychological problems will very difficult get in touch with a social worker or a therapist, on their own initiative.

Based upon four guidelines the Division of Ambulant Psychotherapy of the RNLA has developed a comprehensive set of measures to break down barriers between soldier and therapist in order to make professional help approachable for the soldier in need. All this is done, not to stigmatize the soldier in having psychological problems, but to offer help as soon is possible for the soldier in need and his homefront. These guidelines follow ‘normal’ military principles; like good leadership, group cohesion, and so on. These four guidelines are:

stable individuals,

homefront care,

stress as a normal reaction,

and the Salmon principles.

Policy of the Royal Netherlands Land Army on psychological support by operations abroad.

This policy comprises 10 'steps':

step 1: initial or intake selection for regular soldiers:
by means of among other things personality tests and an interview, aimed at the deployability abroad and psychological fitness, we assess psychological stability and try to filter out the high risk groups.

step 2: education and counselling on stress and social support, preferably by the psychologist who will accompany the unit as a field psychologist when the unit is send abroad. In this process of counselling we also incorporate the homefront. The education consists of training and lessons on stress and especially for key personnel training in debriefing techniques, to apply after calamities have happened and the field psychologist is, for example due to large distances, not available immediately.

step 3: Support by a field clinical psychologist in the area of operations.
Each unit of battalion size has a so called social coordinating committee, already in the barracks in Holland. This committee comprises the unit medical doctor, the chaplain, the welfare officer, the personnel officer (S1), and when the unit is assigned abroad, a field clinical psychologist. The latter has three tasks: he is an advisor to the commander; he supports the key personnel; and he acts as a counsellor or therapists when necessary.

step 4: Family support or homefront care.

The RNLA facilitates the establishment of and guides the so called 'homefront committees'. They comprise partners or parents of soldiers deployed in UN operations, and help each other in difficult times, in meetings and through so called telephone circles.

Of course a sitcen – a situation centre at the Army Headquarters – is available on a 24 hour basis for the family that needs information on the whereabouts of their relatives. Although the RNLA takes initiatives and facilitates with financial help, personnel and so on, it is, and will stay, the responsibility of the partners and parents if they themselves will join the committee of the unit the soldier belongs to.

step 5: Psychological debriefing.

Of course, after each serious incident, the clinical psychologist or the key functionary in the unit will conduct a debriefing. Moreover, a psychological debriefing takes place before the personnel return home after their duty abroad. This is normally done in the area of operations and in the units, but if necessary, with personnel deployed individually as UN monitors for example, debriefing will be done immediately after return to the Netherlands as well.

Eventually, when a clinical psychologist is needed, but is not available in the area of operations (for example because the unit was too small to assign one to) a psychologist or a team will be flown into the area.

During these debriefing meetings written material is handed out on possible delayed effects and how to act if problems arise.

step 6: Reintegration meetings.
8 weeks after returning to the Netherlands the soldiers are invited to take part in a reintegration meeting guided by the social service of the army. This is done in units preferably, but here too individual personnel, again the UN monitors for example, can join these meetings as well. During these meetings the soldiers discuss their adaptation to normal life, in work and family, the so called reintegration process, and the problems they are confronted with. Together they try to find solutions.

step 7: In an active, personal approach, personnel who have been deployed are sent an 'aftercare questionnaire', approximately 9 months following their return. The home front of the servicemen or women also receives a questionnaire. In more detail this step in psychological support will be discussed later on.

step 8: Veteran Care.

Four basic principles guide the policy of the RNLA concerning veterans from operations abroad:

1. veteran care is the responsibility of the army, even though the veterans are no longer part of that army.
2. there should be an active approach, an outreach to the veterans, to survey possible problems and to offer help.
3. the help offered by the army is as accessible as possible. That is, there are no barriers. A veteran in need of support can approach his own psychotherapist, the officer-clincial psychologist who he served with during his duty, even if the veteran has left the army already.
4. there is a good collaboration between the military mental health services, the veterans organisations, and the specialised civilian mental health centres. The Veteran Institute has a central coordinating role in these activities.

Important to mention is the fact that in the Netherlands a veteran is a serviceman who left the army and has been abroad for his term. Other papers on this symposium will give more detailed information about the activities of the veterans care.

step 9: All lessons learned are collected by a special office of the chief of army staff. This relates not only to the experiences in our branch, but also in operations, logistics and so on. Behavioural scientists help to structure the way the information is compiled, and to analyse the data and draw conclusions.

step 10: Last, but not at least, there is the systematic evaluation of all steps mentioned above.

Aftercare questionnaire

As mentioned earlier, research and the experiences of therapists identified soldiers with problems resulting from experiences of a mission. Withdrawl from social contacts, misunderstanding and denial that they have any problem led to psychological problems in which the soldier will not get in touch with a therapist, on their own initiative.

This is why it is necessary for each individual serviceman or woman to be contacted, to determine whether they are encountering problems as a result of their assignment, and to offer them help if they need it.

Against this background, personnel who have been deployed are sent an 'aftercare questionnaire', approximately 9 months following their return. The home front of the servicemen or women also receives a questionnaire. The main purpose of these questionnaires is to offer (after)care to (former) servicemen and women, and their home front. This psychological care will be offered by the Division of Ambulant Psychotherapy.

This active, personal approach is also called **outreach**. Outreach can prevent military personnel having to battle with problems for years, before they finally seek help. It is our experience that the likelihood of successful treatment is greatly enhanced if the symptoms are spotted in time.

This questionnaire includes items regarding to the stressors military personnel experience during their term; a PTSD-survey concerning intrusion, avoidance and hyperarousal experiences; items about mental and physical changes since the mission and adjustments to life at work and at home in the Netherlands; two SCL-90 dimensions; and so on.

In the mid-nineties the Division of Ambulant Psychotherapy has started with this active approach. On this moment almost 30.000 questionnaires has been send out to all servicemen and their home front, started with Provide Comfort in Iraq in the early nineties until now. Table 1 gives some interesting results about this retrospective surveys.

Table 1: Comparison between different periods[1-2].

	Period 1991 - 1996	Period 1996 – 1998	Period 1998 –1999	Period 1999-2000
Surveys returned	N=5035	N=2158	N=1517	N=2029
Response	46%	42%	38%	36%
Most reported serious events:				
- Witnessing human suffering	74.4%	50.9%	44.0%	57,5%
- Shootings (not aimed at the soldier)	79.2%	38.6%	25.6%	38,9%
- Witnessing death/wounded locals	51.8%	22.2%	13.8%	26,9%
- Rejection by local people	47.3%	28.5%	28.4%	25,4%
- Personal danger	43.7%	18.7%	15.2%	16,5%
Readjustment problems:				
- Partial PTSD	20.4%	14.7%	14.0%	16,1%
- Full blown PTSD	4.3%	2.6%	2.0%	2,6%
- Sleep disturbances (SCL-90)	15.6%	12.6%	11.9%	12,2%
- Somatic disturbances (SCL-90)	12.4%	9.8%	7.6%	10,5%
Care:				
- Contacted by telephone	27.0%	16.0%	14.6%	14,0%
- Accepted help	8.0%	3.5%	2.3%	3,1%
- Already treated	2.0%	2.3%	2.1%	2,5%
- Already treated elsewhere	1.2%	1.6%	1.8%	2,5%

Period 1991- 1996: Provide Comfort - ICFY - UNPROFOR - (DI t/m III) - UNTAC - UNAVEM - WEU - etc.

Period 1996- 1998: IFOR2 - SFOR1-2 - MAPE - etc.

Period 1998- 1999: SFOR 3-4-5 - MAPE - UNIPTF - UNFICYP - etc.

Period 1999- 2000: SFOR 6-7 -KFOR1-2 - MAPE - UNIPTF - UNFICYP - etc.

There are high incidences of reported events and readjustment problems for the period 1991-1996. It is the period in which we had to deal with the war in Yugoslavia (e.g. Srebrenica) and Rwanda.. 4.3% of the respondents were diagnosed as having PTSD and for 27% of the respondents there was an indication for problems due to their term abroad.

Reasons for inviting someone for care were:

- reported stressful encounters during operations,
- reported fysical or mental changes,
- welfare or complaints of the partner,
- the respondent asked for an interview with a therapist.

The second and third period gives a decrease in reported serious events, readjustment problems and care. It is the last period 1999-2000 which give rise to all above. A further decline is curved upward, mostly due to the experiences of the Kosovo-conflict.

Over the years we can see a slight decline in response. This is troublesome. In 1999 a non response survey[3] indicated, fortunately, that the main reason for not responding was mostly due to not having problems. Nevertheless, non response is a pity and for the future we must be creative in our efforts how to deal with this negative development.

It became clear from several aftercare studies that military personnel experiencing psychological problems as a result of a mission can indeed be traced by means of the questionnaire and the majority of respondents considered this active approach to be a positive development.

In 1998 a study was conducted by TNO, the Netherlands[4], concerning the physical complaints from servicemen situated in Lukavac in the mid-nineties. From this study it was recommended that every soldier must be monitored psychological as well physical.

Right on this moment efforts are made between physicians and psychologists in sending out an combined questionnaire for signalling medical and psychological problems by servicemen after their return from an operation[5].

Some concluding remarks

An overview of a policy including ten separate steps in which therapists can contribute to peace keeping operations has been presented. This policy, adopted by the RNLA, seems to be effective. There is a decrease in problems, and those psychological problems can be treated at an earlier stage and thus resolved easier, quicker and thoroughly. Above that there is a greater acceptance of the contribution of psychologists in the army. The fact that soldiers too have emotions and can have emotional problems that can be discussed and treated is now a fact of life in the army.

For a better monitoring of the servicemen, efforts are made between physicians and therapists, in sending out a combined questionnaire for detecting medical and psychological problems in an early stage. Future is promising for a better health care system for the servicemen of the Royal Netherlands Land Army.

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SYMPOSIA DISCUSSION - PAPER 27

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Discussor's Name: Surg.Capt Hoejenbos (NL)

Question:
Are there any NATO-guidelines about procedures in the psychosocial field?

Author's Reply:
Not that I know of. If there are guidelines I would be very interested.

Tools and Techniques for Enhanced Health Surveillance in Deployed Settings

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ABSTRACT

Historically, diseases and non-battle injuries have had the greatest impact on military mission accomplishment. In recent years, service members and their leaders are increasingly concerned about potential adverse health effects from environmental exposures occurring far from home. Now, there is also an increasing threat of attacks involving weapons of mass destruction. Prompt preventive action is available for many biological threats and would minimize the number of casualties, but this requires early identification of the attack. Enhanced surveillance techniques may hold the key to such early warning systems. This paper describes recent efforts to incorporate this approach as part of in-theater medical operations.

1.0 INTRODUCTION

Historically, diseases and non-battle injuries (DNBI) have had the greatest impact on mission performance. Consequently, the U.S. Department of Defense (DOD) continuously monitors health events in deployed troops, seeking to minimize the adverse effects of DNBI. For many years, military preventive medicine efforts focused on broad categories of endemic communicable diseases and non-battle injuries. As concerns surfaced about unexplained illnesses among Gulf War veterans and of possible disease clusters associated with other military operations, e.g., the possibility of leukemia among Kosovo peacekeeping forces, DoD efforts increased to better integrate occupational and environmental exposure data with health event data. In light of recent attacks in several countries involving chemical and biological agents, there was a clear need to develop or adapt surveillance systems capable of detecting patterns in health-related data that might indicate community exposures to weapons of mass destruction, especially biological agents. The DoD addressed this need by applying enhanced surveillance techniques to the existing DNBI surveillance system.

2.0 FORCE HEALTH PROTECTION

Force Health Protection is an innovative DoD health care strategy that seeks to maximize health and fitness among U.S. service members, prevent casualties, and ensure the best possible care and management of those casualties that cannot be prevented. Key to this strategy is medical surveillance that seeks to identify disease outbreaks (natural or deliberate) at the earliest possible moment. Critical success factors include timely data flow (same day or multiple times a day), accurate and representative data, suitable analytical techniques with interpretation, prompt reporting of the results to the field medics and to decision makers at various levels of command, appropriate actions to prevent or intervene, and ongoing monitoring for the effectiveness of any

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actions taken. This cycle, called deployment health surveillance, is especially difficult to accomplish under field conditions far from home.

3.0 DEPLOYMENT HEALTH SURVEILLANCE

Deployment surveillance has three phases. Predeployment surveillance occurs in the garrison setting and addresses ongoing health assessments, preventive medicine actions (such as immunizations, serum samples for future analysis, blood type determination, etc.), and timely record reviews and personnel questionnaires just prior to departing to the deployed location. Intradeployment surveillance monitors exposures (disease, occupational, environmental, iatrogenic, etc.) and health events that occur while the service member is deployed; this phase of surveillance is the primary focus of this paper. Finally, postdeployment surveillance focuses on health events that occur later in life, after the service member has returned to his/her home station.

3.1 Intradeployment Surveillance Data Sources and Collection Processes

Table 1 lists the primary health event-related data available from the theater. These sources cover a wide variety of health events, but their usefulness remains restricted due to various limitations. Historically, these limitations included difficulty establishing and/or accessing computer networks and connectivity for a mobile population operating in austere, remote sites, non-standardized information management systems for the various military services, ensuring compliance of complete and accurate reporting under physically hazardous conditions (e.g., "under attack"), and the lack of user-friendly electronic data entry systems. Much progress has occurred in these areas, and several promising systems are undergoing testing in the field.

Data Source	Scope	Timeliness	Limitations
Outpatient Records	All services, but variable collection and reporting processes.	Daily to weekly, but variable compliance.	Primarily broad categories of illnesses as opposed to specific diagnoses.
Inpatient Records	All services, but only at sites with inpatient wards.	Not readily available.	Primarily paper-based reporting and locally managed (limited central access).
Air Evacuation	All services, but only cases evacuated using military aircraft.	Data input real-time.	Severity biased. Causal information lacking.
Mortality Reports	Full autopsy and investigation of all deaths.	Days to weeks.	Severity biased.
Safety Reports	Only cases meeting pre-defined "accident" criteria. Often have extensive information about circumstances and causes.	Days to weeks to months.	Not a medical reporting system. Limited releasability for confidentiality.

Table 1: In-theater Deployment Surveillance Data Sources

3.2 Disease, Non-battle Injury (DNBI) Surveillance Categories

Initial deployment surveillance efforts focused on broad disease and injury categories (see Table 2), primarily because most deployed units had limited access to computers. Using broad categories allowed the deployed

medics to track local health events using paper logs and assigning each visit to the category they thought best based on definitions provided by the U.S. Joint Chiefs of Staff. The system started in the late-1990s and required deployed medical units to send weekly reports to a stateside analysis hub. Such an approach has been very useful from a general preventive medicine standpoint, helping to identify areas needing investigation or special, focused preventive measures. However, it was clear that this approach could not identify possible attacks involving weapons of mass destruction since the categories were so broad (unusual diagnoses would get lost in the "noise" of common events, like upper respiratory illnesses) and the data was 10-14 days old by the time it reached the central analysis hub. Consequently, a decision was made to apply enhanced surveillance techniques, primarily by redefining several categories in a more focused manner and requiring daily reporting instead of weekly.

JCS Deployment Surveillance Category	Examples
Combat/Operational Stress Reactions	Acute debilitating mental, behavioral, or somatic symptoms not explained by physical disease or injury.
Dermatological	Heat rash, acne, fungal, cellulitis, blisters, sunburn
Gastrointestinal, Infectious	Diarrhea, nausea & vomiting, hepatitis (not ulcers)
Gynecological	Menstrual irregularity, vaginitis (not pregnancy)
Heat/Cold Injuries	Hypothermia, frostbite, trench foot, heat stroke
Injuries, Recreational/Sports	Injuries from informal pursuit of personal or unit fitness
Injuries, Motor Vehicle Accidents	Direct consequence of motorized vehicular accidents
Injury, Work/Training	On-the-job injuries or formal unit physical fitness training
Injury, Other	All other injuries not included in above categories
Ophthalmologic	Conjunctivitis, foreign body, corneal abrasion, iritis
Psychiatric, Mental Disorders	All except combat/operational stress reactions
Respiratory	Bronchitis, pneumonia, asthma, sinusitis, otitis, flu, upper respiratory illness (cold)
Sexually Transmitted Diseases	All sexually transmitted infections (chlamydia, HIV, etc.)
Fever, Unexplained	Temperature $\geq$100.5, at least 24 hours, diagnosis unclear
All Other, Medical/Surgical	Any other initial visit not encompassed above
Dental	Any disease of the teeth, gums, and/or oral cavity
Miscellaneous/Administrative/Follow-up	Pregnancy, immunizations, medicine refills, routine physical exams, e.g., visual or hearing screening

Table 2: Deployment Health Surveillance DNBI Categories and Examples

3.3 Enhanced DNBI Surveillance Using Special Surveillance Categories

A group of epidemiologists and preventive medicine specialists established five special surveillance categories that attempt to capture health events most likely to be associated with known chemical or biological warfare threats.

Category	Definition	Threat
Systemic Fever	Unexplained temp > 38C (100.5F) for 24 hours, or a history of chills and fever without a clear diagnosis. Includes flu-like illnesses, with fever and multiple systemic complaints (including cough).	Generic biological agents with ill-defined flu-like illness, e.g., tularemia.
Lower Respiratory Illness	Bronchitis, pneumonia, new onset reactive airway disease, pleurisy, or respiratory difficulty of unclear etiology.	Primarily anthrax, maybe mustard agents.
Infectious GI	Any infection, usually manifest by vomiting and/or diarrhea.	Multi-purpose, but primarily to identify food-related illnesses (could indicate contamination, tampering, or side effects of biological agents, e.g., ricin.
Dermatologic, Unclear Cause	Skin infections, blisters, ulcers, etc.	Smallpox, chemical blister agents.
Unexplained Neuro	Cases of altered consciousness, cranial nerve dysfunction, muscle weakness.	Botulinum toxin.

Table 3: Deployment Health Surveillance Special Categories

3.4 Statistical Analysis and Reporting

Analyzing deployed health event data presents several challenges. Reporting is often erratic as military units move from place to place, interrupting data flow for varying periods of time. Accurate population denominator data is hard to get, so there must be methods for dealing with event counts when accurate rate calculations aren't possible. Health event data of this type, especially in a young population specifically screened for peak health and fitness, are relatively rare events and not normally distributed. Finally, good comparison data are rarely available as local health departments may not have the level of data desired or may not be willing to provide the data. Using rates from other countries or from the unit's garrison setting may well mislead the analyst as the environmental conditions, levels of endemic disease, and other unique factors likely differ significantly from place to place. Past analysis of deployment health surveillance data from two military sites 20 km apart showed substantially different disease rates despite apparently similar populations, weather conditions, surrounding industrial exposures, etc. The best approach is to determine site- and population-specific comparison rates at the earliest possible time. Based on these requirements, the decision was to modify the graphs of current/past experience[1] employed by the Centers for Disease Control and Prevention with reporting of significant national medical event patterns.

3.4.1 Current/Past Experience Graphs (CPEG)

The CPEG approach compares the number of reported cases (or rates) for a specified time period (day, week, month, year) and compares it with historical data (e.g., over the previous month, compared to the same period of time in each of the last five years, etc.). The analytical method uses the 'z-score' derived from the normal approximation of the exact Poisson calculation for rare events. This method has the advantage of standardizing the scale, which allows data for various outcomes (health events), locations, and periods of time to be graphed together.

$$p - value = \frac{e^{-\lambda} \cdot \lambda^{x}}{x!} \qquad z\text{-}score \cong \frac{\sqrt{obs} - \sqrt{exp}}{\frac{1}{2}}$$

Exact Poisson calculation **Normal approximation**

Obs=observed value, Exp=expected value

Figure 1: Statistical Equations for Poisson and Z-score Calculations

The normal approximation[2] is simpler than the exact calculation and has the added advantage of being easy to calculate using standard Microsoft® software applications like Excel® and Access® that are more readily available in deployed settings and eliminates the need for expensive advanced statistical software packages. The normal approximation is sound when sample sizes are large. If the population (denominator) fluctuates by more than 20% during the identified time period, expected counts can be adjusted to reflect the difference. Figure 2 is a sample CPEG. It is graphically dense, summarizing large volumes of information on a single, easy to read, slide. Increases and decreases from the expected baseline are readily apparent with red indicating excursions significant at the 0.01 level while green indicates values within the expected historical range. This tool is specifically designed to be highly sensitive. A high proportion of false positives is acceptable given the disastrous potential outcome of missing a serious disease outbreak or attack. A side effect of this sensitivity is that gradual changes in the event counts or rates will not trigger a threshold violation. A single statistical analysis is not enough. An industrial process control approach fills this gap.

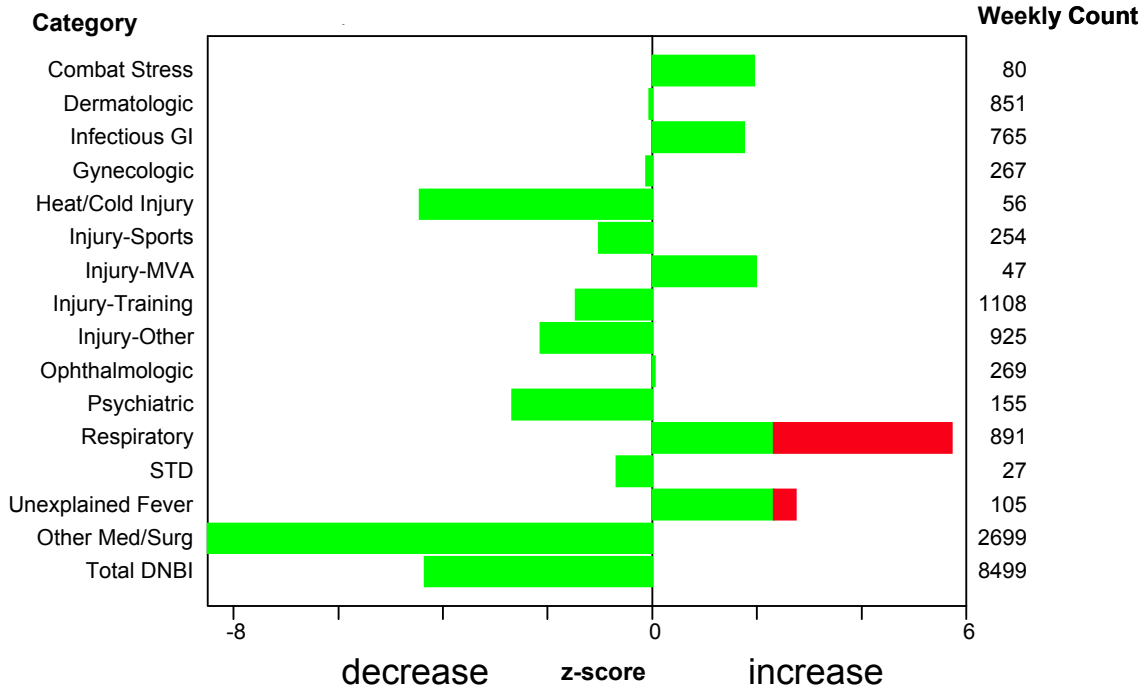

Figure 2: Presenting Data Using a Current/Past Experience Graph

3.4.2 Process Control Graphs

Figure 3 illustrates a process control method for documenting variations from a mean over a specified time period, six months in this case. There are two threshold levels depicted. These thresholds are sometimes referred to as upper control limits in quality control literature. One is an intermediate alert level (yellow line) established as two standard deviations (S.D.) above the six-month mean while the other is a higher level alarm (red line) established at three standard deviations above the mean. Though lower control limits are also used in manufacturing (e.g., assembly line) control process analyses, there is little utility to including them in health event analyses where decreases in health events are almost always beneficial. The desire to identify outbreaks at the earliest opportunity must be tempered by limited resources. Public health investigations divert staff from other duties, so rather than immediately responding to every excursion above an alert or threshold line, the following tiered approach is used to determine when to request a focused investigation:

- Any time the count or rate exceeds the red alarm level (i.e., >3 S.D.)
- When two out of three consecutive data points exceed the yellow alarm level (i.e., >2 S.D.)
- When five out of six consecutive data points exceed the 6-month mean

The example in Figure 3 shows two of these conditions, one alarm in the first week of May and one alert in the first week of July. Seeing six months of results at a glance also makes it easier to follow seasonal trends and other long-term variations.

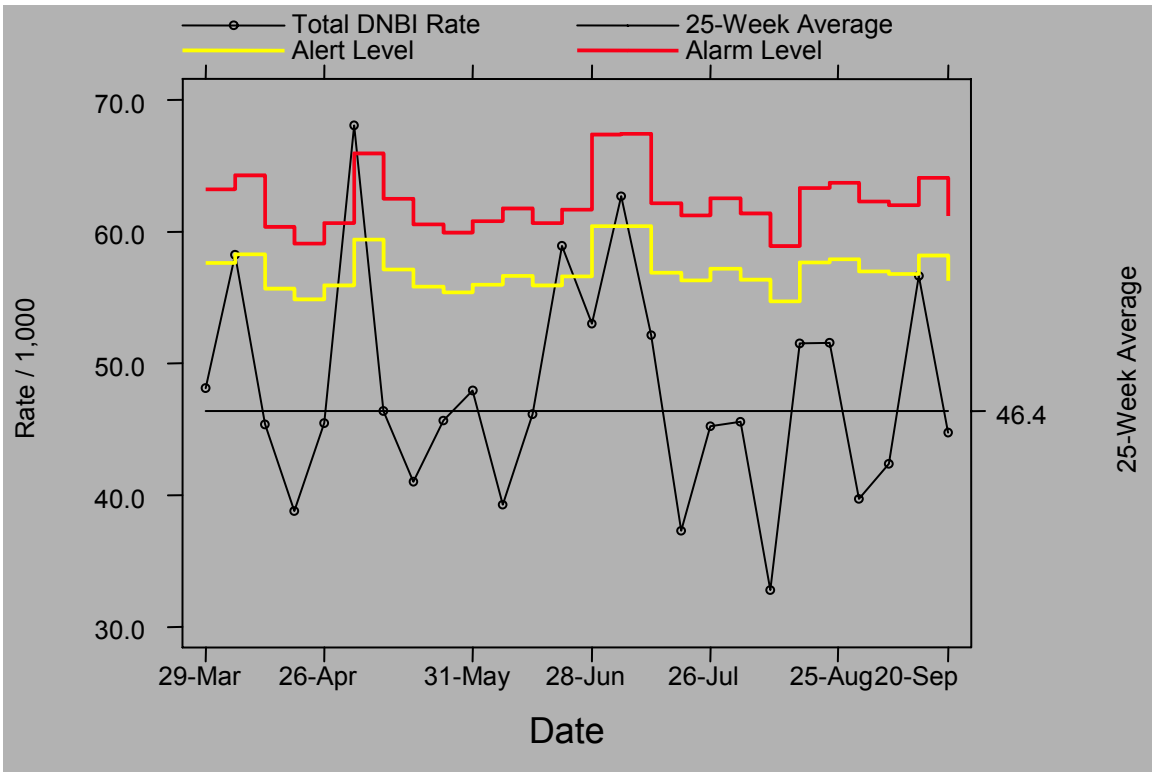

Figure 3: Example Process Control Graph

3.4.3 Final Analysis, Interpretation, and Distribution

Using the CPEG and the process control graphs together provides a more complete picture. Figure 4 is an example of such a paired output. Unit 322 on the CPEG shows a red bar indicating a small, but statistically significant increase in febrile respiratory illnesses (FRI) for the week of 14 March 2003 compared to the previous four weeks. However, the process control chart provides some reassurance in that the 14 March rate is actually below the two standard deviation alert level. Consequently, the on-site public health staff may reasonably choose to postpone any aggressive investigation and continue to follow the results the next few days to see if there is a continuing upward trend. In cases where the remote analysts are unsure of the significance, they contact the field medical staff to ensure that they are aware of the situation and ask them to provide additional information based on their local evaluation. These comments are then added below the graphs and combined into a report available for review on a classified Internet site. The final reports often include site-specific graphs as well as aggregate reports on a regional, operational, or component service level.

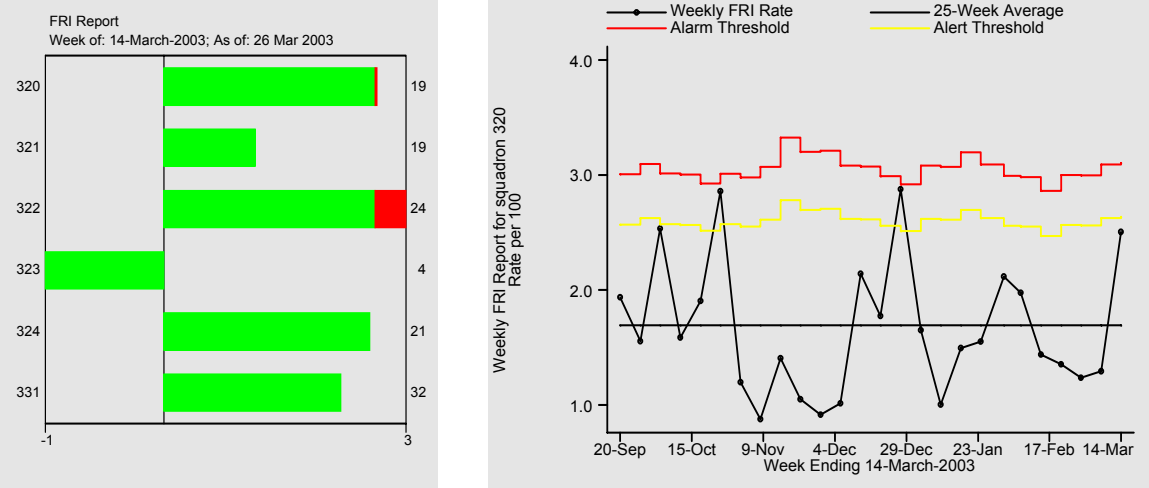

Figure 4: Complementary Use of CPEG and Process Control Charts

4.0 FUTURE DIRECTIONS

This paper focuses on deployed health event surveillance, especially as one method to help identify unrecognized attacks involving weapons of mass destruction. The described methods hold promise, but there remain a number of limitations and a need to validate and compare these techniques with other methods, especially in regard to possible long-term health outcomes related to exposures occurring during deployed military operations. Some areas to address include:

- Data gaps
  - Important data categories remain difficult to obtain, such as comprehensive battle injury data; centralized electronic data for inpatient care occurring in-theater, e.g., hospitalizations and surgeries, access to electronic laboratory data including the emerging field versions of various rapid screening tests (PCR, microarrays, various biomarkers), etc.
  - Lack of coding for causal/situational factors in injuries, accidents, and fatalities
  - Long-term outcome registries for reproductive health, cancer, etc.
  - Timely environmental and occupational exposure data
  - Accurate geospatial location data for both individuals and exposures (e.g., plumes)
- Dealing with data overload
  - Adapting artificial intelligence and/or neural nets to help parcel large data streams into manageable portions for human analysts to interpret while balancing sensitivity and specificity
  - Integrating diverse data streams, e.g., exposure data with health outcomes
- Standardization of data collection, analysis, and reporting processes across the DoD
  - Validating and refining statistical methods, disease category mappings, alarm threshold levels, etc.

5.0 CONCLUSIONS

The U.S. Department of Defense has made significant progress in establishing health screening and surveillance systems to cover a service member's entire military career, and even after the individual leaves military service. More and more electronic data are becoming available, but more is not necessarily better. Inconsistent and incomplete data capture, human errors (e.g., inaccurate coding) and other limitations complicate interpretation. It remains critical to validate the many proposed health surveillance methodologies in order to identify those data sets and statistical approaches that will provide the best value. Ongoing health surveillance is one of the key pillars of Force Health Protection and will help the U.S. meet its heartfelt obligation to protect the health of American military service members, their families, and the communities with which they interact around the world.

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[1] Stroup DF, Wharton M, Kafadar K, Dean AG. Evaluation of a method for detecting aberrations in public health surveillance data. Am J Epidemiol 1993;137(3):373-80.

[2] Hirsch RP, Riegelman RL. Statistical First Aid. Interpretation of Health Research Data. 1992. Boston; Blackwell Scientific Publications.

SYMPOSIA DISCUSSION - PAPER 28

Authors Name: Col Cox (US)

Discussor's Name: Prof. Dr von Restorff (GE)

Question:
Do you collect environmental data at the same time?

By environmental I mean weather etc and activity because both may influence health.

Author's Reply:
U.S. DoD does collect extensive occupational and environmental exposure data, including meteorological data. A central archive exits, but data flow is not completely established across the military services. Once the system is functioning, we will begin to review health events, both acute and long-term, against known environmental exposures to see if there are unrecognized relationships.

Authors Name: Col Cox (US)

Discussor's Name: Surg.Capt Hoejenbos (NL)

Question:
1) How can it be prevented that people are counted twice or more?
2) Are other personnel than military in the system?

Author's Reply:
1) Guidelines tell medics to report initial visits in the respective categories. There is a separate category for follow-up visits. Errors do occur. Planned electronic data collection systems will help eliminate these mistakes.
2) The deployed health surveillance system only applies to active field operations. It captures data from all people presenting for care at the deployed medical treatment facility, including allied/coalition members and deployed U.S. civilians or contractors. Similar, standardized surveillance systems will apply to the in-garrison (home-front) setting.

Medical Situational Awareness in Theater Advanced Concept Technology Demonstration Project Proposal

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ABSTRACT

There is a need for greater medical situation awareness in theater and for greater integration of theater medical information into the net-centric rapid communications structure envisioned by DoD. Current information systems are not well integrated and do not provide optimal trend analysis or alerts that identify health and readiness risks that can be rapidly used by Combatant Commanders and by Joint Command Medical Departments. This project is designed to apply maturing data collection, communication, and computer-based decision aids to solve important medically-related military problems. An Advanced Concept Technology Demonstration (ACTD) Project is proposed to solve the problem of medical information fusion and communication for medical situation awareness in the theater.

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

1.0 INTRODUCTION

Combatant Commanders lack timely, complete, actionable health information for operational decision-making, thereby putting troops at unnecessary risk for illness or injury and jeopardizing force strength and morale. There is a need for greater Medical Situation Awareness in theater and for greater integration of theater medical information into the ForceNet (i.e., a net-centric, rapid communications structure) structure envisioned in current DoD planning. Current information systems are not well integrated and do not provide optimal trend analysis or immediate warning alerts that identify risks that can be readily be used by Combatant Commanders and by Fleet Surgeons. Advanced Concept Technology Demonstrations (ACTDs) are designed to exploit maturing technologies to solve important military problems. An Advanced Concept Technology Demonstration Project is proposed to solve the problem of medical information fusion and communication for medical situation awareness in the theater.

ACTDs were first introduced in the Department of Defense in 1994 and were envisioned to be a pathway for mature technology to be rapidly introduced to solve new or persistent problems. Generally, an ACTD identifies a problem, maturing technologies that can address the problem, and brings together key partners. A competitive process is employed with final selection of ACTDs made by vote of Joint Combatant Commanders.

This proposed Medical Situation Awareness in Theater ACTD, brings together key sponsors, operational commanders, and medical research assets. These include:

- The Primary Sponsor, the Office of the Assistant Secretary of Defense, Deployment Health Support Directorate.
- The Technical Manager, United States Army Medical Research and Materiel Command.
- The Lead Investigative Center, the Naval Health Research Center.
- The Lead Operational Command, Pacific Command Surgeon.
- Transition Manager, the Joint Medical Information Systems Office.

The proposed project supports Joint operations by providing Joint commanders and staff with enhanced knowledge of the health readiness of their forces by generating information on emerging medical threats and health-related trends during deployment, so that when warranted, operational plans may be adjusted on a real-time basis. It also supports the Joint Force commander by providing medical threat and trend information during pre-deployment as well as for application to follow-on forces prior to their deployment into the Joint Operational Area. Another significant benefit of collecting and archiving this information in a standard manner is that it can be used to develop support plans for future operations and to support post-conflict studies and research.

2.0 PROBLEM

Current field medical data systems and domains that do not connect well include: medical intelligence, occupational and environmental hazard monitoring, chemical and biological threat monitoring, trauma reporting, disease and non-battle injury data, personnel unit and location data, and other critical data. These data are generally collected by different agencies, often sporadically, and are not universally shared, making it an impossible task to sort, understand, and generate actionable knowledge within operational timeframes.

3.0 CANDIDATE TECHNOLOGIES

This project recognizes that the concepts in ForceNet require near instantaneous collection, communication, analysis, and advanced computer-driven decision aids that can provide an integrated picture of the theater of operation. Mature technologies exist today that can begin to solve the problem. The use of these technologies must be closely linked to concepts of operation that take advantage of new technologies. Each of the Services has developed interim data capture capability in medical and non-medical areas pertinent to this project. The analysis of the disparate data and the reporting of results will allow protective measures to be implemented and factored into the medical and operational status of a deployed Joint Force. Several key elements include: Potential technology contributors include:

- Net-centric Communications and Control
- Service-specific Field Medical Data Systems
- Common Medical Operating Picture
- Joint Medical Workstation (JMEWS)
- Transportation Command Regulating and Evacuation System (TRAC2ES)

See Figure 1 below.

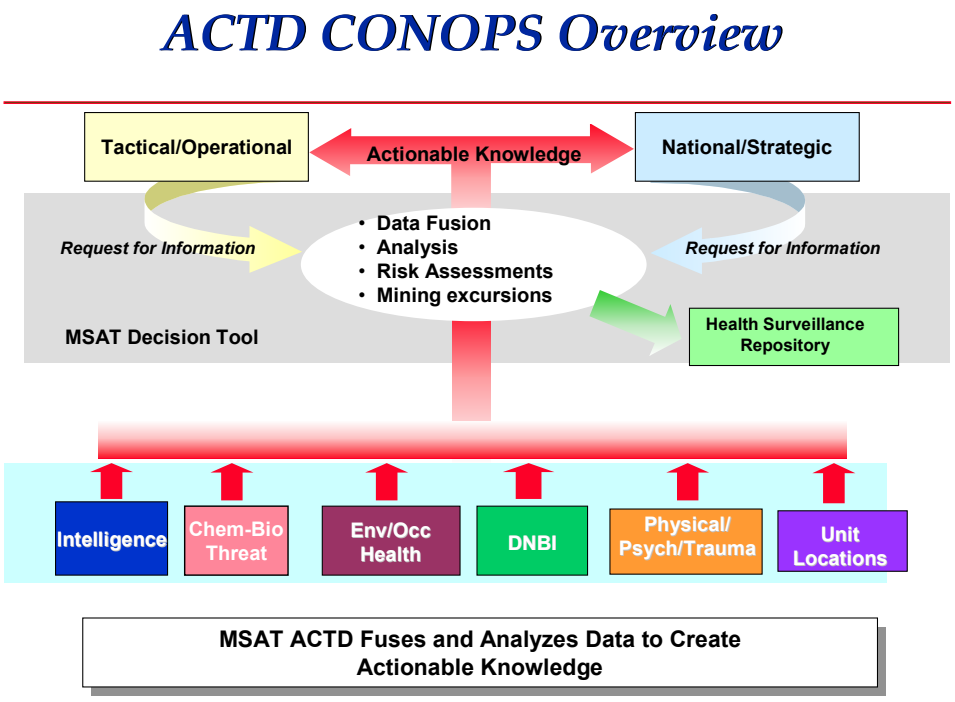
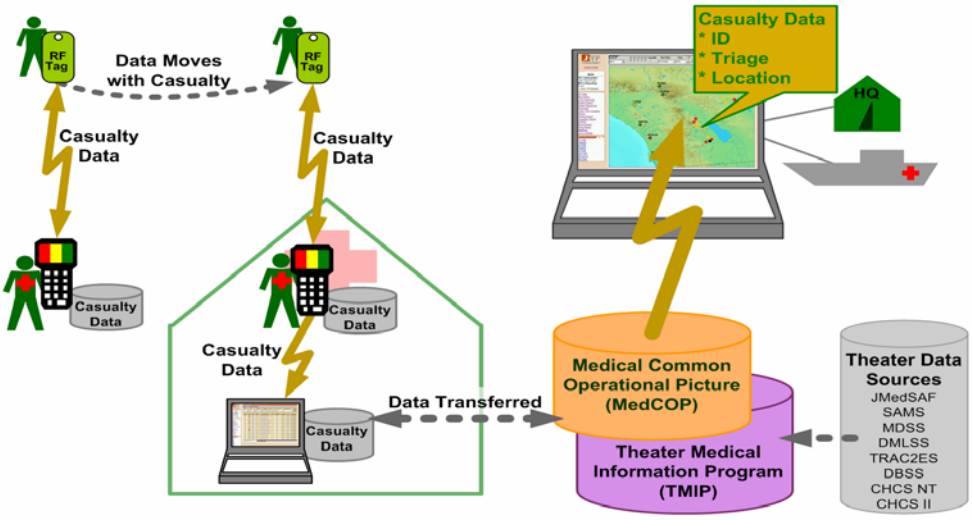


Figure 1: ACTD CONOPS Overview.

Currently a number of commercial technologies exist or are being developed that may support the project and they will be exploited; they include: Gemini/Modernized Integrated Database; fusion applications; artificial

intelligence capabilities; web-enabling technologies; sensor and point-of-use data capture technologies; and technologies for the capture and analysis of physiological changes.

The project will fuse current and emerging technologies and apply artificial intelligence and computerized decision support systems to transform collected, scattered data into timely, actionable information and knowledge. Secondarily, as a by-product, the project will archive the data to establish a DoD repository for health surveillance information that provides reach back capability to Joint Force and Combatant Commanders, Joint Staff, the Services, the Office of the Secretary of Defense, and others. The project Concept of Operations (CONOPS) overview is depicted in Figure 2.



Example of Medical Data Flow in Theater

Figure 2: Medical Data Flow in Theater.

4.0 PROJECT SCHEDULE

During the first year of the project, the project will refine the architecture and identify new and sufficiently mature technologies for possible insertion or integration into the project concept. By the last quarter of the first year we will stand up the Medical Situational Awareness System and begin the first of a series of field trials to determine the utility of the technologies and the concept for the fusing, and trending, and archiving of information. These activities will continue through the second and third years (FY06 and FY07) with a full concept demonstration of the capabilities of the technology and associated tactics, techniques, and procedures in a Joint exercise or operation scheduled to begin in FY07.

5.0 IDENTIFICATION OF POTENTIAL MEASURES OF EFFECTIVENESS AND PERFORMANCE

Measures of effectiveness will be developed for:

- Ability and time required to identify, communicate, and react to immediate and emerging medical and environmental health care threats.
- Ability to combine, communicate, and process data from currently disparate systems.
- Ability to provide situational awareness of health hazards to Joint Force commanders.
- Degree to which units and personnel assigned to those units can be identified with exposures to occupational and environmental hazards.

Products with military utility will remain for COCOM use; the long term funding and support mechanisms currently identified will ensure operational capability remains throughout the extended user period. Additional residual capabilities include: continued refinements of the Medical Situational Awareness System; continued ability to populate the DoD Health Surveillance Repository and to share data with an emerging user base; capability to verify and adjust physiologically-based models that form the backbone of decision systems; and the availability of data for multiple longitudinal analyses (analysis of long-term health outcomes, new doctrine, enhanced training regimens, etc.).

This project will use spiral development to introduce and assess various capabilities over the period of field trials. Feasible technologies will be spun off for early field operational evaluation and implementation. Successful technologies and operational concepts may be transitioned early to an acquisition program of record for adoption and force structure fielding. The targeted program of record is the Joint Medical Information System. At the conclusion of the project, final recommendations on military utility will be provided to Combatant and Medical Commanders in theater.

The Career History Archival Medical and Personnel System (CHAMPS): An Epidemiological Data Resource for Force Health Protection

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LONGITUDINAL EPIDEMIOLOGIC STUDIES OF DOD PERSONNEL USING THE CAREER HISTORY ARCHIVAL MEDICAL AND PERSONNEL SYSTEM (CHAMPS)

The Career History Archival Medical and Personnel System (CHAMPS) is a comprehensive database that provides an individually based longitudinal record of career events and hospitalizations from the date that an individual's military service began until the date of separation or retirement. Most routinely collected administrative or medical data are a collection of discrete events that are compiled and updated in monthly files. CHAMPS organizes these routinely collected cross-sectional files into an individually based narrative history that is organized by the event date and type of event. CHAMPS chronologically tracks career events that include deployments, duty station or ship assignments, job designations, foreign port visits, changes in the number of dependents and many other types of personnel and military career events. Outcomes include medical events such as hospitalization discharge diagnoses, coded using an International Classification of Diseases Code (ICD), HIV testing results, service separation (including type of discharge), or death with ICD or external cause code. CHAMPS' flexible architecture also allows addition and analysis of data from dependents of service members. CHAMPS provides a rapid, cost-effective method for defining cohorts of military personnel and following them longitudinally for subsequent medical or personnel events. CHAMPS enhances the DoD's ability to conduct epidemiologic research and provide force health protection for active-duty forces and has been used to study a wide variety of potential exposures and health outcomes of military importance.

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

1.0 INTRODUCTION

The concept of force health protection depends on the ability to readily ascertain environmental factors that may be related to deployments or occupational exposures and examine subsequent medical and personnel outcomes, including hospitalizations (1). The Career History Archival Medical and Personnel System (CHAMPS) is a comprehensive database that provides an archival career and medical history for active-duty military personnel organized at the level of the individual in a chronological narrative format. Key career and military events are coded and recorded in order by event date. In this way, a military service member can be tracked from accession to service separation. Because of this unique design, the CHAMPS system is ideally suited for use in conducting longitudinal observational studies using a variety of career-related variables to define exposure. CHAMPS has been used to study factors associated with the incidence of HIV seroconversion (2-7), including risk of HIV seroconversion following visits to foreign ports by Navy personnel (8). CHAMPS has been used to study the incidence of first hospitalization for a variety of chronic and infectious diseases and injuries in active-duty personnel potentially associated with military occupation or duty-station assignment (9-21). Studies of chronic disease include incidence of outcomes such as diabetes (9), Hodgkin's disease (10) and non-Hodgkin's lymphoma (11) and testicular cancer (12) and melanoma (13). CHAMPS was used to conduct studies of leukemia in both active-duty personnel (14) and their children (15). Injury studies include studies of the incidence of heat stress (17), eye injury (18), carpal tunnel syndrome (19) and others (20). Data sets from CHAMPS have also been used as a basis for epidemiologic modeling and have been used to define populations at risk in the military according to demographic or service characteristics. CHAMPS data sets have been used to compare incidence of malignant neoplasms in military personnel with that experienced in the civilian population (9-14), and have also been developed to determine the expected occurrence of adverse pregnancy outcomes aboard U. S. Navy ships (21).

2.0 METHODS

CHAMPS is created and updated from a wide variety of military data sources. Detailed personnel data are currently obtained from the Defense Manpower Data Center (DMDC) in Monterey, CA, USA. This information includes key demographic and career events including name, SSN, date of birth, race, gender, home of record, service and date of accession, military training schools attended, qualifications and testing results, changes in pay grade, rank and duty station, and dependent changes. A variety of potentially adverse events such as positive drug testing results, AWOLs, desertions, demotions and discharges are also obtained.

The design of CHAMPS is analogous to that of a relational database that organizes medical and personnel data into an individual narrative format composed of a chronologically arranged history of events in the individual's military career. Like commercial relational databases, CHAMPS' architecture allows any number of records to be incorporated for a particular individual. CHAMPS' unique event-driven architecture combines data from numerous sources in the DoD into a single coherent chronologically organized record. It uses advanced commercial software that allows rapid quality assurance and editing, integration of new data, and manipulation of the approximately five million career histories that it contains. CHAMPS is updated on a quarterly basis to reflect the latest career changes and medical events during an individual's military career.

The largest current source of health outcome data used by CHAMPS comes from the Department of Defense Tricare Management Activity (TMA) Standard Inpatient Data Record (SIDR) which includes admissions to military hospitals. The SIDR electronic record identifies hospital discharge diagnoses in the International Classification of Diseases (ICD) format. CHAMPS captures up to eight discharge diagnoses for each individual inpatient hospital admission to a DoD medical treatment facility. A second source of

hospitalization data that has been used with CHAMPS to ascertain health outcomes among dependents is the Health Care Service Record (HCSR), available from TMA. This is used to ascertain DoD-reimbursed hospitalizations of beneficiaries outside military medical treatment facilities. The search procedure used is extensive, but is limited to hospitalizations in military or civilian hospitals receiving payment for medical care from DoD. It does not include hospitalizations not reimbursed by DoD, or any not yet reported in the SIDR and HCSR databases. CHAMPS currently contains no information on outpatient medical treatments or events, but these data will soon be added using on the Standard Ambulatory Data Record (SADR) database of outpatient visits to DoD medical treatment facilities available from TMA which contains outpatient records going back to 1996. Some additional data elements contained in CHAMPS include results of HIV testing for all military personnel and dates and locations of foreign port visits by Navy personnel assigned aboard ship. Foreign port visit events are derived by combining duty station assignment codes for specific ships obtained from DMDC with ship movement data provided by the Chief of Naval Operations (CNO).

CHAMPS can be used to support a variety of epidemiological study designs. It can be used to define cohorts by age, race, gender, time-period and occupation or duty-station assignment. The population at risk in these cohorts can be expressed as the number of individuals in a group over a particular time-period or as the number of person-years in a specific group. This information can then be used to determine an expected number of events in a particular military population, such as the number of expected cancer diagnoses in a particular occupational group (11-14) or the number of adverse pregnancy outcomes expected aboard a particular group of ships (21). CHAMPS is also well suited for use in long-term longitudinal studies to determine person-days of exposure in various occupations or duty stations and has been used to create extracts for use in proportional hazards regression studies. An example of some particular source files used for CHAMPS and how they enter an individual CHAMPS narrative structure is illustrated in figure 1. Examples of types of extract files and methods for analysis are also shown.

3.0 RESULTS

Results from several studies that were conducted using the CHAMPS system are presented below. These studies illustrate the advantages of CHAMPS' individually based chronological file structure.

3.1 FOREIGN PORTS OF CALL AND RISK OF HIV ACQUISITION

The US Navy visits ports on all continents and many islands of the world, many of which have been reported to have a high prevalence of human immunodeficiency virus (HIV). CHAMPS was used to carry-out a nested case-control study to examine the relationship between visits to the 100 foreign ports most frequently visited by the navy and risk of HIV seroconversion (8). Prior to this study, there had been no link between personnel assigned aboard ship and ship movement information. DMDC had information on individuals assigned to ships, but no ship movement data. CNO had ship movement data, but no information concerning the identities of personnel assigned aboard ship. CHAMPS was used to create this link at an individual level by linking ship assignment data to ship movement data, resulting in creation of a foreign port visit event code. A total of 813 HIV seroconverters were matched to 6993 seronegative active-duty controls by age, race, sex, occupational group, homeport, and year of test. The unique longitudinal structure of CHAMPS was used to identify cases who had an initial negative HIV test and foreign port deployment, followed by a positive HIV test. Odds ratios of seroconversion associated with visits to foreign ports showed no statistically significant excess risk of HIV infection for navy personnel after visits to any of these foreign ports. Despite the mobility of the US Navy and the large variation in HIV seroprevalence rates throughout the world, navy personnel generally did not appear to be acquiring HIV infections abroad.

3.2 A Model for Adverse Pregnancy Outcomes Aboard Navy Ships

The goal of this study was to model the incidence of ectopic pregnancy and spontaneous abortion if pregnant women were allowed to remain aboard ship during the first 20 weeks of gestation while at sea during deployments. Ectopic pregnancies and other pregnancy complications at sea can be life-threatening events. Data sources included information from shipboard medical departments, an enlisted personnel survey, and the NHRC CHAMPS system. The overall pregnancy rate was 19 per 100 woman-years (95% confidence interval, 18-20), based on the complement of women assigned to participating ships. The population of women assigned to all Navy ships and specific ship-types was determined using CHAMPS. If pregnant women routinely were to remain aboard ships at sea during deployments through their first 20 weeks of pregnancy, it was estimated that approximately 9 ectopic pregnancies and 40 spontaneous abortions would occur aboard ships at sea annually.

3.3 A 27-Year Historical Prospective Study of Sarcoidosis and Other Pulmonary Disease

This study used the CHAMPS system to examine long-term trends in incidence rates of pulmonary sarcoidosis and other lung diseases in a large cohort of Navy personnel and to evaluate the relationship between sarcoidosis and other lung disease to Navy occupation (22). CHAMPS was used to determine person-years at risk in particular Navy enlisted occupational groups and the average annual populations at risk over the entire study period. Incidence rates of first hospitalizations were calculated for black and white male Navy enlisted personnel on active-duty between 1975 and 2001. Specific occupational groups may have had greater exposure potential. First hospitalizations included cases of sarcoidosis (n = 674), asthma (n = 3,536), emphysema and chronic bronchitis (n = 1,103), respiratory conditions due to fumes and vapors (n = 61), and pneumoconiosis (n = 51) observed in approximately 10 million person-years of active-duty service. Overall hospitalized sarcoidosis incidence rates per 100,000 were 24.9 for black males and 3.5 for white males (black/white ratio= 7.1). Annual incidence rates in blacks declined markedly during the study period (figure 2). This decline could not be explained by an increase in incidence rates of the other major lung disease categories examined. Occupational associations were present in blacks and whites. Black ships servicemen (23 cases) and aviation structural mechanics specializing in structures (12 cases) had more than twice the expected incidence rate compared to all blacks, and white Mess Management Specialists (15 cases) had twice the overall white incidence rate. Occupational associations suggest the possibility that sarcoidosis may have a previously unrecognized occupational component.

3.4 Investigation of a Potential Leukemia Cluster in a Town Near a Naval Air Station

The Nevada State Health Department identified an apparent cluster of 16 cases of acute lymphocytic leukemia (ALL) that were diagnosed in children and teenagers in Fallon, Nevada, USA. Fallon is in Churchill County, which is the location of the Fallon Naval Air Station (NAS). For this reason, the Nevada State Epidemiologist asked the Navy for help in locating other possible leukemia cases in children of naval personnel who may have left the state. Data resources of the DoD Deployment Health Research Center at NHRC were used to identify potential cases of leukemia among Navy families. CHAMPS was used to determine whether family members were ever stationed at NAS Fallon (15). More than 400 incident cases of acute lymphocytic leukemia were identified among active-duty Navy and Marine Corps personnel or their dependents from 1 January 1997 to 15 March 2001 using SIDR and HCSR hospitalization data. Duty station assignments to NAS Fallon were identified from the CHAMPS career history using a sequential search of over 12 million records. No active-duty person who developed leukemia during the study period had a history of assignment to NAS

Fallon. Two leukemia cases were identified in children of active-duty personnel who had been assigned to NAS Fallon. These two cases had been known to the Nevada State Health Department and were already part of the previously recognized cluster.

3.5 Basic Epidemiological Modeling and Research

CHAMPS was used to perform a methodological study that compared results of three epidemiological study-designs carried out to examine the association between military occupation and incidence of a particular cancer during the same time interval. The study compared results of a nested case-control study using conditional logistic regression (7,600 individuals) with results of a baseline cohort study (right censored only, with 1.9 million individuals and 10 million person-years of follow-up), and results of a full cohort study (ongoing recruitment and right censored observations, with 4 million individuals and 18 million person-years of follow-up). The study provides an analysis of results of the nested case-control design using conditional logistic regression compared with two types of cohort studies using proportional hazards regression and will provide a basis for understanding the relative power and sensitivity of these three approaches to detection of epidemiological associations and the evaluation of the strength of the associations.

4.0 CONCLUSIONS

CHAMPS is a unique data system that is well suited to longitudinal epidemiological investigations in the DoD. Its individually-based flexible architecture allows it to be readily adapted to support a wide variety of specific investigations of high military relevance. CHAMPS enhances the DoD's ability to conduct epidemiological research in support of force health protection.

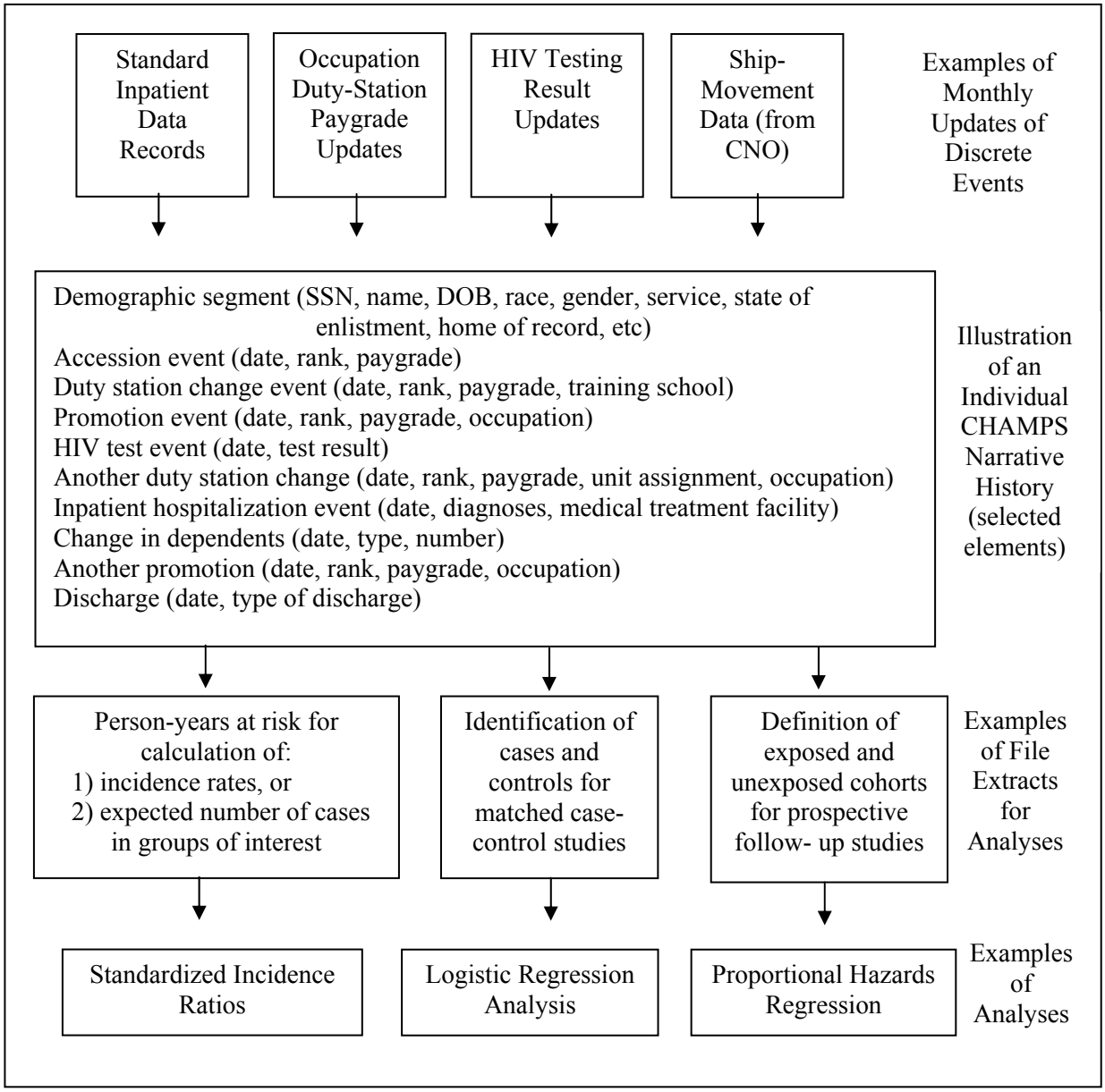

Figure 1: Examples of selected source files for CHAMPS, illustration of a CHAMPS narrative history, and examples of file extracts and types of analyses

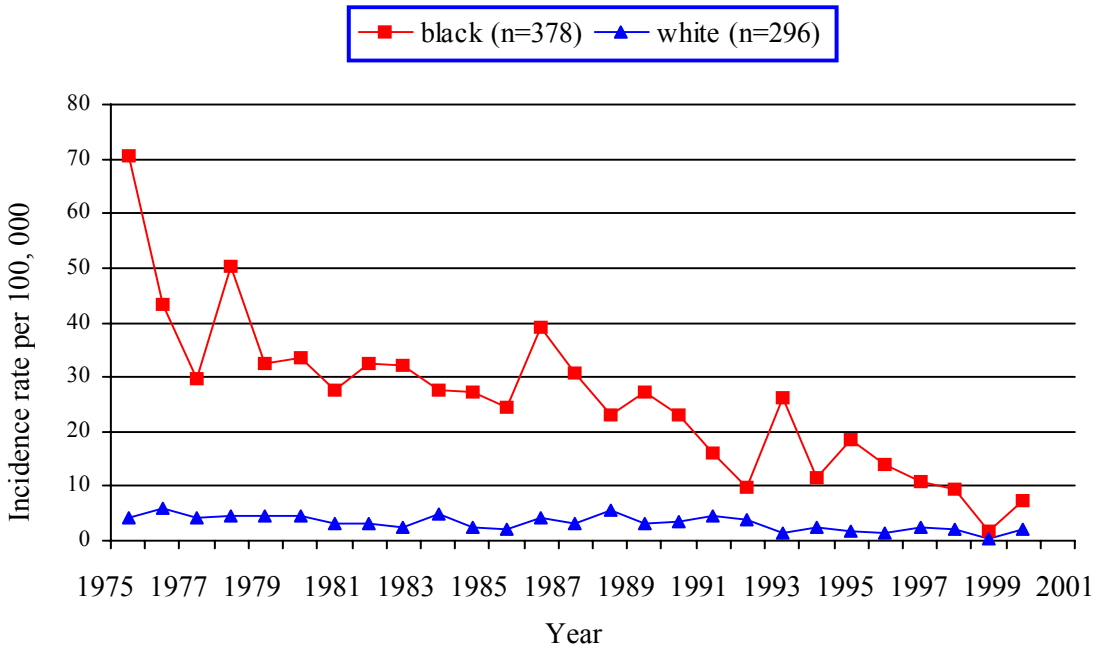

Figure 2: Average annual sarcoidosis incidence rate per 100,000, active duty naval enlisted men by race and year, 1975-2001

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The Millennium Cohort Study

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For the Millennium Cohort Study Team

ABSTRACT

Introduction and Relevance

Concern has been raised in the decade following the Persian Gulf War of 1990-91 that military service, and operational deployments in particular, may lead to long-term health problems. Chronic, multi-symptom illnesses have been especially challenging to assess in post-deployment troops. Retrospective epidemiologic analyses have been both difficult and costly for the US Department of Defense.

Rationale

US policymakers, academicians, and veterans groups called for a prospective cohort study to better assess the relationship between chronic health problems and military service.

Methods

Beginning in 2001, the Millennium Cohort began enrollment of a stratified random sample of more than 140,000 service members, with the intention of following their health for up to 20 years. Cohort members will provide self-reported data on medical symptoms, functional status, and health-related behavior every 3 years. New and existing sources of objective data on health and health-related exposures, both during and after service, will be leveraged and linked to the cohort at regular intervals over the next 20 years.

Results

Nearly 80,000 service members enrolled in the Millennium Cohort in the first phase of the study. Participants are demographically well representative of the target population. Enrollment surveys demonstrate greater than 95% completion rates. Initial data capture from all sources, including internet-based surveys, is rapidly progressing.

Conclusions

The Millennium Cohort Study has been successfully launched. This 20-year project is expected to better define the long-term health of US military service members. The study's ability to prospectively evaluate both objective and subjective health status, in relation to deployments and other occupational exposures, will make results of high interest to both military and civilian public health professionals.

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

1.0 BACKGROUND

In 1990 and 1991, the United States (US) deployed more than 700,000 military service members to the Persian Gulf region as part of Operation Desert Shield and Operation Desert Storm. The troops' engagement was brief and very successful by all military standards. In the wake of these deployments, however, some service members raised concern about medical problems that they attributed to their service. Their medical concerns included a wide range of problems, including injuries, infectious diseases, mental health challenges, and ill-defined, chronic, multi-symptom illnesses. Over the next ten years, countless hours and more than $1 billion (US) were expended exploring the issue of "Gulf War-related illnesses."[1,2]

Past studies of post-deployment health problems were all retrospective in design. Epidemiologically, such designs are challenged by confounding, selection of appropriate control groups, response biases, and recall biases. In responding to the concerns of deployers from the Gulf War, clinicians, policymakers, and researchers were frustrated by such study designs. In 1999, the US Institute of Medicine issued a report calling for a large prospective study to better define the long-term health risks of military occupations and deployments.[1] The US Department of Defense also formally recognized the need to coordinate such a prospective evaluation of its service members.[2] In response, the Millennium Cohort Study was developed.[3]

2.0 METHODS

The Millennium Cohort Study investigators include epidemiologists from all branches of the US military services as well as the US Department of Veterans Affairs. The original protocol was developed with considerable input from leading cohort study researchers. A select Scientific Steering and Advisory Committee (SSAC) was commissioned, composed of premier academic epidemiologists to closely guide the study over time. The Millennium Cohort SSAC also includes representatives from leading US veterans' service organizations, such as the American Legion, in order to ensure that veterans' concerns are represented in scientific discussions.

The primary objective of the Millennium Cohort Study is to determine if risk factors related to military service, such as occupations, deployments, and other exposures, are associated with the development of chronic disease. Outcomes include objective medical diagnoses, such as those made during a hospitalization, as well as subjective symptoms and functional status. To assess such outcomes, investigators rely on serial survey evaluations of cohort members, and linking to many objective health-related databases. Objective data sources include automated healthcare encounter data, both inpatient and outpatient, from both military and civilian facilities, pharmacy data, immunization data, occupational data, exposure data, disability data, and mortality data. It should be noted that not all such databases were available or complete in the era immediately after the 1990-91 Gulf War.

Power to define a wide range of illnesses requires a large sample size. Approximately 140,000 service members will ultimately be enrolled in the Millennium Cohort. Nearly 80,000 were enrolled in the first phase in 2001. An additional 40,000 will be enrolled in 2004, and 20,000 more will be enrolled in 2007. All will be followed through 2021. In total, the study will include more than 2,500,000 person-years of follow-up. Original participants were invited from a random sample of more than 250,000 men and women in the US military in 2001. The sample was stratified to include adequate representation of those with a past history of deployment, women, and those in the US National Guard and Reserve forces. Invited personnel in 2004 and 2007 will be drawn from random samples of service members with fewer than two years of service at the time

of enrollment. This will ensure that the Millennium Cohort will adequately represent current forces and recent deployments.

The enrollment survey was developed with expert consultation, using standardized instruments whenever possible. It contains a consent form and a comprehensive battery of questions evaluating past medical problems, mental health issues, symptoms, functional status, health-related behaviours (such as tobacco and alcohol use), and self-reported occupational and environmental exposures. The survey may be completed on paper and takes approximately 30 minutes. Importantly, participants may also enroll and complete their surveys over a secure internet site, www.millenniumcohort.org. On-line completion takes less than 30 minutes and receipt of data by the investigative team is almost immediate. Participants enter unique identifying numbers to access the survey on-line, and their private information is managed with the highest levels of security. Enrolled participants will be asked to complete a follow-up survey every 3 years for the duration of the project.

At the inception of the Millennium Cohort, it was recognized that maintaining the engagement of participants over the life of the study would be challenging. Most US military members spend only 3-5 years in service, so follow-up includes much post-service civilian time. Potential participants are invited to join the cohort using modified Dillman methods that include alternating postcards and survey mailings. Email invitations, carefully phrased to not appear to be "spam" email, are also used. Marketing consultation is sought to develop efficient and effective text for such invitations. Citing endorsement of the study by the US Deputy Secretary of Defense has been helpful in reassuring some participants of the legitimacy of the project. To maintain engagement and foster a sense of cohort identity, enrolled participants receive "thank you" postcards every six months, on US Memorial Day (May) and Veterans Day (November) holidays.

It is recognized that collection of participants' data over the internet site has many advantages. This mode of data collection saves time and tremendous resources in postal contact. It also results in cleaner data that require no scanning or verification prior to analyses, saving many more study resources. Given these advantages, participants are encouraged to provide data on-line through incentives (cost-saving initiatives), in the form of a hat, T-shirt, or phone card, in appreciation for their use of the secure internet site. The Millennium Cohort tokens they receive serve the secondary purpose of enhancing cohort identity and motivation to continue long-term participation. In addition, use of the internet site allows sharing of general study information and updating of contact information. This is considered important for this mobile population of young adults.

3.0 RESULTS

A pilot test of Millennium Cohort enrollment, focused at a 1% random sample of potential participants, was completed in May 2001. Invitations for enrollment in the full cohort were initiated in July 2001. The first enrollment year was marked by challenges related to the terrorist attacks on the US on September 11, 2001. These events resulted in rapid mobilization and deployment of many military members, making them more difficult to contact. In addition, anthrax contamination was discovered in the US postal system in October 2001, resulting in many months of mail delays and non-delivery throughout the country. Despite these challenges, repeated attempts to contact potential participants resulted in more than 77,000 members consenting and enrolling in the Millennium Cohort. Greater than 50% of participants provided their information over the secure internet site, www.millenniumcohot.org.

To date, the study team has performed extensive analyses of data quality. Question-by-question completion rates for the survey, on both the internet and paper versions, have been evaluated. Despite the length of the

survey and personal nature of the health questions, no survey question had a completion rate lower than 80%. Most questions were completed by more than 95% of participants. Question-by-question completion rates for surveys received over the internet were consistently higher than paper survey completion rates.

The study team has been concerned about response bias, that is, how well do Millennium Cohort participants represent the entire US military. Multivariable logistic regression analyses have compared responders to non-responders, including all available demographic variables in statistical modeling. Investigators have found that responders are statistically slightly more likely to be older, more highly educated, of white race/ethnicity, female, married, officers, in the Reserve of National Guard forces, and to work in the fields of communications, intelligence, health care, technical support, or functional support. It is notable that the odds ratios associated with these demographic differences ranged from 1.1 to 1.7. When health outcomes are analyzed, investigators will be able to control for these small demographic differences in multivariable modeling.

The study team has also been concerned that survey responders may have different baseline health characteristics than non-responders. To date, an analysis of healthcare utilization in the 12 months prior to 2001, among both respondents and non-respondents, has been conducted using data from the pilot study. Survey responders averaged 3.3 days of healthcare use and non-responders averaged 2.9 days of healthcare use. The difference was not statistically significant in multivariable modeling. Although this implies that that there is little or no difference in baseline health between Millennium Cohort participants and others in the US military, continued analyses will explore this important issue among the full cohort.

Finally, the study team has wanted to ensure that differential access to the internet did not create a response bias. Multivariable logistic regression modeling, comparing internet respondents to paper respondents revealed that internet respondents were slightly more likely to be on active duty (as opposed to Reserve or National Guard forces), male, non-black race/ethnicity, married, between 25 and 35 years old, high school graduates, and working in the fields of electronics, communications, intelligence, functional support, or other technical specialty. Since the odds ratios associated with these differences were small (1.1 to 1.5) the differences do not appear large enough to bias study results.

Investigators will continue to examine these issues, and other important questions related to data quality and representativeness, in preparation for long-term evaluation of health outcomes in the cohort.

4.0 CONCLUSIONS

The Millennium Cohort study has been successfully launched. This ambitious 20-year project, following nearly 140,000 participants, is expected to better define the long-term health effects of military service. The study's ability to prospectively evaluate both objective and subjective health status, in relation to deployments and other occupational exposures, will make results of high interest to both military and civilian public health professionals.

Collaborative studies have already been proposed by both federal and academic institutions, and guidelines for evaluating collaborative protocols have been developed. The first health outcomes-based studies will include:

- Evaluation of mental health, prior and subsequent to the terrorist attacks of September 11, 2001,
- Evaluation of anti-malarial medication use and potential relationship to long-term health challenges,
- Evaluations of potential health changes associated with deployments to US Operation Enduring Freedom and Operation Iraqi Freedom.

The Millennium Cohort is a large, resource-intensive effort. External oversight is needed and welcomed by the investigative team. The primary protocols, and all sub-study protocols, are designed with external consultation. Multiple human use (ethical) review boards evaluate the project at least annually. The funding organization coordinates external scientific review by the American Institute of Biological Sciences approximately biannually. The US Armed Forces Epidemiological Board, which includes premier scientists from academia, acts as a public health advisory body to the study team. In addition, the study-specific Scientific Steering and Advisory Committee, comprised of leading US epidemiologists and veterans service organization representatives, provides invaluable insight to investigators.

This project will answer health questions about military occupations, and it is likely to serve as the foundation for many future epidemiologic studies. Its prospective design, size, and ability to capture a full range of health-related exposures and outcomes will make results valuable to both military and civilian communities. The results of the Millennium Cohort study are likely to resonate in public health for years to come.

ACKNOWLEDGMENTS

Co-investigators on the Millennium Cohort Study include: Paul Amoroso, MD, MPH, Edward Boyko, MD, MPH, Gary Gackstetter, DVM, PhD, Gregory Gray, MD, MPH, Tomoko Hooper, MD, MPH, and James Riddle, DVM, PhD. Research assistants on the study team in San Diego, California include: Gia Gumbs, MPH (coordinator), Suzanne Clark, Thomas Corbeil, Lesley Henry, Susan Hume, Sheila Jackson, Nick Martin, Robert Reed, MS, Besa Smith, MPH, Tyler Smith, MA, Steven Speigle, Timothy Wells, DVM, PhD, James Whitmer, and Sylvia Young, MD, MPH.

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SYMPOSIA DISCUSSION - PAPER 31

Authors Name: Cdr Ryan (Speaker Cdr Young) (US)

Discussor's Name: Surg.Capt Hoejenbos (NL)

Question:
1) Is there an interaction between questionnaires used in study and the normal post-deployment questionnaire?
2) Is pre-deployment information used?

Author's Reply
1) The normal post-deployment health assessment questionnaire is independent from the Millennium Cohort Study questionnaire. Anyone who deploys is required to complete the post deployment health assessment questionnaire. Only a randomized sample is invited to complete the Millennium Cohort Study survey.
2) There is hope to link in the future with pre-deployment information from the recruit assessment program surveys which do collect baseline health information in military personnel upon entry into the service and this data can be used to augment pre-deployment information.

Authors Name: Cdr Ryan (Speaker Cdr Young) (US)

Discussor's Name: Dr Reifman (US)

Question:
What is the nature of the questions in the study (e.g. subjective, qualitative, etc).

Author's Reply:
The questions come from validated survey instruments such as the Patient Health Questionnaire (PHQ), SF-36 Medical Outcomes Short Form, and Patient Checklist (PCL) and are self-reported and subjective. Analyses linking with hard objective data such as information from the standard inpatient data record on hospitalizations and the standard ambulatory data record on out-patient visits are planned.

Authors Name: Cdr Ryan (Speaker Cdr Young) (US)

Discussor's Name: Dr Foster (US)

Comment on the Ryan/Young talk:

Millennium Cohort (MC) was designed as a formal, "Framingham" type, long term epidemiological study with funding from both the DoD research account and the DoD health care system. The questionnaire data collection is initial with periodic updates driven by date not by military status. The self-reported data from questionnaires are supplemented by data from visits to health care providers. This dual data gathering challenge will be ongoing for the next 20 years. Epidemiology research projects seeking to use MC data sets will be independently proposed, peer reviewed and funded. The "collaboration guidelines" provide guidance on how researchers can gain access to MC data for research.

Health Monitor Instrument 6 Months Post Deployment

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ABSTRACT

In order to gain more insight in the prevention of health complaints of military personnel after deployment, the Dutch Ministry of Defense has appointed the Prevention and Health Department of the Netherlands Organization for Applied Scientific Research (TNO) to develop a new Health Monitoring Instrument (HMI). The aim was to draft a compact questionnaire, which should provide an indication of the general health condition of the individual soldier. By using the HMI exactly 6 months after the end of the deployment and by editing some questions explicitly about the deployment, it should be possible to use the questionnaire for monitoring both the general health condition and possible problems related to the deployment. Individual health profiles and derived group tables can be generated automatically by using the SPSS program. It is possible to draw up general health profiles with accessory risk profile. In case individual soldier scores a 4 or higher, it is advisable to investigate if special care, or intensifying present care, is desirable. With this health surveillance instrument it is not only possible to monitor the individual health status, but also the health status of groups.

1.0 INTRODUCTION

In order to gain more insight in the prevention of health complaints of military personnel after deployment, the Dutch Ministry of Defense has appointed the Prevention and Health Department of the Netherlands Organization for Applied Scientific Research (TNO) to develop a new Health Monitoring Instrument (HMI). This instrument should preferable be used together with the already existing psychosocial questionnaire.

The urge to do more on health monitoring was originated on the several complains after the UN missions in Cambodia, Uganda en Bosnia in the nineties of last century. The minister of Defense installed several commissions (Mrs. Thiesinga was chairman in several of this commissions) to advise about improvements in the post deployment care. Since then a better structure for care for veterans was organized in the Netherlands, for instance a separated Institute for Veterans. One of the advises was also to make a HMI.

2.0 MEANING

There were 3 meanings formulated for the project. The first was to make a monitoring instrument in the form of a small questionnaire of no more than 10 pages. The maximum of pages was necessary because the questionnaire should be combined with the existing psychosocial questionnaire and a too much paperwork could result in a too big non response.

Paper presented at the RTO HFM Symposium on "NATO Medical Surveillance and Response, Research and Technology Opportunities and Options", held in Budapest, Hungary, 19-21 April 2004, and published in RTO-MP-HFM-108.

The instrument was meant to be used for the total health situation of every military post deployment.

De second meaning was to develop an individual scoring system on which base for every single military a short health profile can be made. If a too high health risk profile is scored, the person can be invited for a further consult to determine if more or specific health care is necessary and or wanted.

The third meaning was to make a standard rapport in which on group level the health situation is described. This rapport can be used to compare different deployments or other parameters.

3.0 THE WAY OF WORKING

The make a new questionnaire an inventory was made of information on health of military and the way that screenings instruments were used to get that information. A selection was made to get only those questionnaires, which could or should predict health problems in the post deployment situation caused by the deployment. The literature recherché was validated with the opinion of the defense public health experts.

Literature was also found in the special health problem investigations that followed specific deployments (Cambodia and UNPROFOR), and done by university institutes or TNO. The post deployment medical en social rapport's of the Royal Netherlands Navy and Royal Netherlands Army were used and the advises and suggestions that were given in the past to develop a generic health monitor instrument..

It appeared to be necessary to get more information on screening and on detection of health care problems. So, a Medline-search focused on health of military, Gulf War Syndrome, Chronic Fatigue Syndrome and generic instruments to monitor the health situation of groups of employers. On base of this information it was decided which indicators were the most appropriate to monitor the health situation of military. Baseline for the development of the HMI-instrument was to use as much as possible standard scales and questionnaires, that are used in the civil community or in the military.

This makes it better possible to use the results of the questionnaires and compare them to the standards. The following surveys and questionnaires are (for instance) used for selection:

- The Veteran survey that was done by order of the Ministry of Defense (Bramsen et al, 1997),
- The post-deployment survey of the psychological department of the army (AIH)(Flach & Zijlmans, 1998),
- The Cambodia complain study (De Vries et al, 1998; 2000)
- The periodic health surveys that are used by the occupational health department of the army.

Because of the specific target of the HMI-instrument questions are added to gain insight in the different health risks during deployment, that can have influence on the health perception of a military. The different civilian health questionnaires and the selected instruments, with their possibilities and problems are discussed in the original rapport (TNO, 2003).

4.0 THE HMI-QUESTIONNAIRE

The Netherlands Defense organization wanted to have an instrument, which could give as well individual information as information on group level. On both levels there should be insight in:

- Prevalence of health problems post deployment
- Changing in (number and sort) health problems post deployment;
- (experienced) exposure to risk factors (not only focused on stress or psychological factors, but all factors that can potential influence health);
- the (social medical) health care needs, that are related to this.

The instrument had to be as compact as possible, to make it easier to combine the existing psychosocial questionnaire and keep the non-response as low as possible. The combination should not exceed 10 pages. In the psychosocial questionnaire several items are asked, which are also necessary for the interpretation of the health situation. That is the case not only for name, addresses, demographic and background variables, but also the PTSD-list and exposing experiences. Those items are also part of the HMI results.

All those considerations were part of the discussion and at the end a selection was made in 10 clusters for the HMI:

- General health situation (inclusive functional disorders).

- Experienced heath and experienced relation to deployment

- Diseases and long-lasting health problems

- Acute health problems and infectious diseases

- Non-specific complains/post deployment complains

- Fatigue

- Sleeping problems

- PTSD

- Functional disorders

- Extra load (burden) factors

These ten clusters are related to health and possible exposure of risks. Above that some questions concern the use of healthcare and the need for healthcare.

In the original rapport the considerations are explained around the decision to involve certain questions or not. In every cluster there was a decision about the most appropriate questionnaire. If this was a general (civilian) questionnaire there was a discussion if this instrument was also applicable in the military setting. If necessary there were made some adjustments to make in better usable in the military setting, but without changing the original formulation so much, that the results are not anymore to compare with the standards.

4.1. The individual health profile

In table 1 is written which scores are used as normal in the different clusters. The following starting points per cluster are used for the score:

- First the scores are used on base of references in articles. As much as possible the official and standard scores in the literature is used, if possible based on a military population.
- Above that per cluster a second score is made based on remarks with respect on content (unless there was no real reason to detect).
- If the score exceeds the lowest point this is called “a risk factor”. If the highest point is exceed there is “a signal”.
- If there is no reasonable theoretical base for the threshold for “risk factor” or “signal” (for instance: there is no or not yet information about references) the threshold is decided on by the experts committee using the references in the pilot.
- If there was no usable external norm for a separate indicator it was decided, that in the pilot no more than 20% should exceed the threshold point. It was kept in mind, that the pilot population had a quiet “normal“ deployment without special risks or expected health problems.
- Even if the scores for the threshold points were scientifically based, the criteria are adjusted on base of the results in the pilot. This is applicable for the individual clusters of questions and scales but als the combinations.

For the amount of exceeding of the thresholds (“risk” and “signal”) a score can be counted per person. In the expert group a decision is made on the preferable action, that should follow than. Till now the opinion is, that in such a case the professional (doctor or psychologist) will make contact by telephone. In the conversation both can agree upon the need for further contact with a special medical doctor or psychologist. Before this call is made there is tuning between medical and psychological expertise.

The results of those calls can be used in the evaluation of the HMI and decisions about adjustment of the thresholds.

To calculate the individual score the following principles were used:

- Score = 1 if the “risk threshold is passed, but not the “signal” point.
- Score = 2 if the “signal” point is passed.
- Per cluster gets because of that the score 0 (no exceeding of any threshold), 1 (passing risk-border) or 2 (passing signal border).
- All the clusters are counted together and that is the total score.

If the total is higher than 4 the military are qualified for follow-up. This is the case if:

- Minimum of 2 signals; or
- Minimum 1 signal in combination with a minimum of 2 risk factors or,
- Minimum 4 risk factors without any signal.

On base of the references it can be concluded, that cumulating of health problems is a better predictor for post deployment complains that the existence of a specific health problem. In the present way of counting it is assumed, that problems only need more active care, if more clusters are involved. If a lower threshold is used the group of "high health risks" will be to big, and the possibility of coincidence (for instance because a temporary problem like the flu) will be too great.

4.2. Relation between HMI and Psychosocial questionnaire

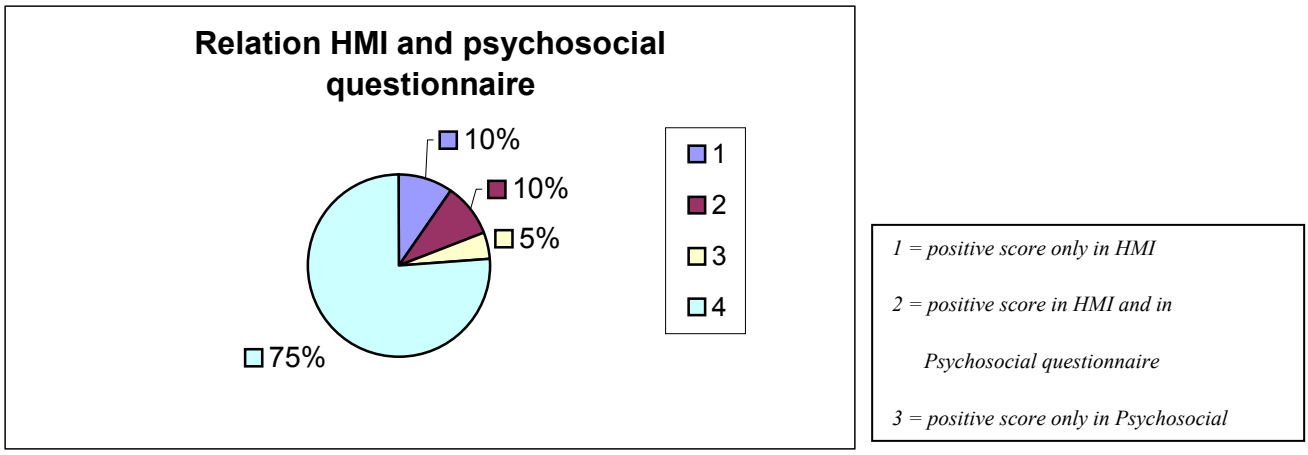
In the graphic (illustration 1) is shown what the relation is between the original psychosocial questionnaire and the new HMI. The relation between both post-deployment healthcare indicators is substantial and significant ($p<.001$). This is also caused because some psychosocial indicators were used in the HMI. Also in general, it is known, that there is always found a substantial correlation between psychological and other health indicators in surveys like this (Wessely, 2001; De Vries, 2001; Mulder & Reijneveld, 1999).

In the graphic it can be seen, that of the 50 persons who have a positive indicator for further suggestion for the post-deployment healthcare on base of the HMI-algorithm exact the half (25 persons) will also be positive on base of the interpretation of the psychosocial questionnaire.

The agreement on the group for which no further post deployment healthcare is necessary is much larger. On base of the HMI-protocol 209 of the 259 persons (80,7%) have so low amount of health problems reported, that they don't need further contact. In almost 95% of the cases, the psychological opinion is the same: no further healthcare proposal is needed for them. In 5% of the cases (12 persons) the psychologist, decided on base of the different answers on the list, that a telephonic contact was needed.

When the 2 adjustments are compared, the conclusion can be made, that the introduction of the HMI in the Defense post deployment period will lead to more military, for whom there is an (possible) indication for post deployment (health) care. To conclude if that is really the case other investigations are necessary.

ILLUSTRATION 1



5.0 THE GROUP MONITORING

The rapport about the results on group level is made in the format of a compact monitoring rapport. The information (graphics and tables) are generated right away from the statistic software, that is used for the data-analyses (SPSS 11.0). That is the reason that in short time it is possible the produce a basis monitoring rapport. This is done to give quick insight in the most important health relevant characteristics of the deployed population. On this way it is possible to decide if (and if so: on which specific part) further investigation or healthcare is necessary. In every page, a health cluster is discussed. First, there is a short introduction in the items in the cluster. Than a table is shown in which the prevalence of health problems in that cluster is given. This is combined with the percentage of the deployed population in which the criteria are exceeded. To get insight in the differences between subgroups 4 relevant background variables are divided: age (in 3 groups), rank (soldier, NCO, officer), type of contract (fixed short versus lifetime employment) and the if the contemporary deployment was the first deployment of those military. These differences between the subgroups are validated on significance.

A problem for interpretation of further differences is the small amount of people in the subgroups. No validated conclusions can than be thrown.

Reporting of other items like adjustment problems and adaptation problems and opinion on preparation and post deployment care and items related on the home front are no subject of the group monitoring rapport if the HMI, but are the responsibility of the department, that deliver the psychosocial questionnaires.

6.0 PILOT

In the summer of 2002 there was a pilot of the combined questionnaire (the regular psychosocial questionnaire and the new HMI). This was a group of 855 military who were deployed in a peacekeeping mission SFOR (roulation 10) in 2001 to Bosnia. This group military consisted mostly of Army (90%) together with some of the Royal Military Police (10%). They got the questionnaire a half year after they had returned.

Because the pilot was taken in a rather difficult period (summertime) the group that didn't react got a new second chance. Both questionnaires were put in the computer at the psychological department of the Army. Both parts of the questionnaire got a unique identifier, via which combination of the two parts was possible. The anonymised HMI-information, together with some special psychosocial items (which were important to make a interpretation for the HMI) were given to TNO-PG. TNO made the analyze and rapport of the information. In the future, this work can be dome in the Defense organization without TNO.

7.0 CONCLUSIONS

From the pilot the conclusion can be made, that the HMI in general has met the demands. Because the obliged maximum of pages (6 pages) there were some concessions in the possibility to ask more questions on certain health aspects. Some questions seemed to give only a little extra information. If questions add only a prevalence of less than a few percent it can be decided to skip those questions if a more compact questionnaire is necessary, unless the question is integral part of a validated questionnaire. The contemporary HMI-list has to be seen as a prototype. Not only will changing the psychosocial list change the HMI but also should special risks during deployment have influence on the actual questionnaire. Moreover, not in the least: the results of scientific work and experiences with the list should develop the instrument.

Also a further integration of psychosocial questions and health questions is necessary to minimalism the overlap, as it is necessary to harmonize the work of the psychosocial and (social) medical departments.

It can already be advised, that 2 questions should be added, which are concentrated on the concept of 'Un-Met Needs'. This is the need for help or care, although this help or care is not in fact asked by the person. The questions about this are formulated in the Nemesis Survey (Bijl en Ravelli,1998).

Evaluation of the made normscore of 4 points is necessary. It seems to give a good criteria for the decision to suggest more "care" to the military. This can better be validated if a randomized trial is made of selected people who got more care and (if possible) a selection of persons who didn't get a suggestions of more care.

If The HMI questionnaire is used on an other moment than 6 months post deployment, the profiles (scores) and questions have to be adjusted. On this moment in some questions the period of the validated questions happens to be synchronized with the 6 month deployment and the 6 month post deployment period for instance long-lasting health problems, use of healthcare system, un-met-needs)

It is necessary to try to optimize the response. In the pilot the response was only 35%. The low, and sometimes selected response has consequences for the conclusions that can be made about the health situation of the total group. The conclusions (and the suggestion to give for more health care) for the individual respondent is in fact to trust. But still it has to be taken in account, that a substantial part of the deployed group, especially those with a higher risk for health problems (low rank, low educated and young military with a short time contract) do not in the same ratio take part in this health care monitor.

May be the wish to give optimal individual healthcare (for which the personal identity is necessary) conflicts with the wish to get an objective insight in the mental and physical health of the deployed military. Possible a "non response evaluation" can give more information about the background. Also can be thought about the possibility to give feed-back about the (eventually anonymized)l results and advises of the questionnaire.

From the perspective of individual healthcare seems the relative low response not a very great problem, because everyone who wants the join, can join. However, the problem to keep in mind is, that the non-response group exists especially from people with a higher amount of complains.

The data from the questionnaires of different deployments should be put in a databank to get a military reference. Than it will be possible to compare different deployments and different (sub-) groups. Then it will be possible to detect patrons an signals in health complains in time.

The questionnaire and the results of the pilot are available (in Dutch) via the authors.

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TABLE 1

Cluster	criteria	Eventual extra condition(s)
Cluster 1A: Physical health general (SF-12)		
Criteria risk factor	PCS < 50	
Criteria signal	Non	
Cluster 1B: Mental health general (SF-12)		
Criteria risk factor	MCS < 50	
Criteria signal	Non	
Cluster 2: Experienced health		
Criteria risk factor	Experienced health rather bad or bad OR score now < 6: OR diminishment of score between 'pre deployment' and 'during or after deployment' >=1.5: Or diminishment score between 'pre deployment' and 'contemporary score >= 1.5	Only if the diminishment of > 1.5 point is possible caused by the deployment.
Criteria signal	Experienced health rather bad or bad	
Cluster 3: Long-lasting health problems		
Criteria risk factor	Minimum of 1 long-lasting or heavy complain in last year, which:	Still is there and If no medical doctor is consulted for that in the last year.
Criteria signal	Minimum of 1 long-lasting or heavy complain in last year, which:	Still is there and If no medical doctor is consulted for that in the last year. Was not there in the year before deployment.
Cluster 4: Infectious diseases		
Criteria risk factor	Often (3 times a year or more) of 1 of the 4 health problems and also:	If no medical doctor is consulted for that; If the problem is post deployment more often than before.
Criteria signal	Non	
Cluster 5: Non- specific illness complains		
Criteria risk factor	If since the departure to the past deployment there were a minimum of 3 complains (disregard the situation before)	
Criteria signal	If since the departure to the past deployment there were often a minimum of 3 complains (disregard the situation before), if:	Minimum of 2 complains of these we not regular existing before deployment; Or if the problem was more often there in the last year than in the year before deployment. (in the case of infectious diseases).
Cluster 6: Fatigue		
Criteria risk factor	Score >= 32 on 8 items from the CIS-Fatigue	
Criteria signal	Score >= 37 on 8 items from the CIS-Fatigue	
Cluster 7: SCL-90 Sleeping problems		
Criteria risk factor	Norm score of SCL-90 (3 items >= 6 (m) or 7 (f)	If there was also a regular sleeping problem during or post deployment.

Criteria signal	Non	
Cluster 8: PTSD		
Criteria risk factor	Definition of partial PTSD: Score >= 1 on re-experiencing or Score >= 3 on Avoidance or Score >= 2 on Hyper arousal.	
Criteria signal	Definition of full blown PTSD: Score >= 1 on re-experiencing and Score >= 3 on Avoidance and Score >= 2 on Hyper arousal.	
Cluster 9: Functional disorders		
Criteria risk factor	Post deployment functional disorders (regardless of sort or how bad) in regular work	
Criteria signal	Post deployment functional disorders (regardless of sort or how bad) in not to heavy work	
Cluster 10A: Experienced physical burden and bother during employment		
Criteria risk factor	During deployment inconvenience caused by 3 or more named factors (physical strain, climate, exposure to chemicals).	If minimum of 1 of these had influence on the health.
Criteria signal	During deployment regularly inconvenience caused by 3 or more named factors (physical strain, climate, exposure to chemicals).	If minimum of 1 of these has (according to the person) still influence on the health.
Cluster 10B: experienced mental stress during deployment		
Criteria risk factor	If the deployment was experienced as “rather or very” thrilling, threatening, or powerless	If this was the case in minimum 1 of 3 questions.
Criteria signal	Non	
Cluster 10C: experienced mental stress, Life Events		
Criteria risk factor	More than 1 rather touching to very touching situation in the period around the deployment.	Without life event related to own health.
Criteria signal	Non	

Health Surveillance among Dutch Military Personnel during the United Nations Mission in Eritrea and Ethiopia

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SUMMARY

Dutch troops participated in the United Nations Mission in Eritrea and Ethiopia from December 2000 until June 2001. All personnel (1089) received information on health risks, with emphasis on anti-mosquito personal protective measures. Malaria chemoprophylaxis comprised weekly mefloquine or daily doxycycline. Immunisations included meningitis A+C and yellow fever. When necessary boosters for diphtheria, tetanus and poliomyelitis (DTP), hepatitis A +B and typhoid were administered. During deployment the health surveillance showed a mean non-availability of 0.41 %. The mean consultation incidence was 18.6 per 1000 men days. The main diagnostic groups included: orthopaedics & injuries (24.9 %), dermatology (22.3 %) and miscellaneous (20.6 %). Two cases of p.f. malaria were diagnosed; 1 year after return 5 cases of plasmodium vivax malaria had shown up. In a post deployment questionnaire 76.6 % indicated consistent use of malaria chemoprophylaxis, 15.4 % inconsistent use, while 2.8 % never used at all. The use of bed nets scored: 31.6 % always, 42.8% irregular and 25.8 % never. About 30 % of the bed nets and 10 % of the uniforms proved to be impregnated. Consistent use of DEET was reported by 1.5 %, inconsistent use by 36.9 % and no use 61.6 %. Overall the non-availability remained very low but compliance to personal protective measures needs continuous attention.

INTRODUCTION

A Dutch military battalion participated in the United Nations Mission in Eritrea and Ethiopia (UNMEE) from December 2000 until June 2001. Because of possible health risks the troops were routinely monitored during and after deployment. Moreover it follows the recommendations of several political and scientific reports based on the experience with post deployment complaints among Dutch troops after several operational deployments. This study presents the results of the health surveillance during the mission and the outcomes of a post deployment questionnaire.

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STUDY POPULATION AND METHODS

Study population

The Dutch troops were part of a combined Dutch Canadian Battalion (NECBAT, n=1643) and were stationed in five campsite locations divided over the central part of Southern Eritrea (4) and Northern Ethiopia (1). Dutch troops comprised 1089 personnel (38 females) with a mean age of 28.4 years who stayed in theatre from 171200 until 030601. On departure all personnel were fit for duty.

Preventive measures

All personnel received oral and written information on health risks several weeks before departure. Additional attention was paid to personal protective anti-mosquito measures such as use of long sleeves and trousers between sunset and dawn, insect repellent (diethylmethyltoluamide: DEET 30 %), and permethrine impregnated uniforms and bed nets. On this latter it should be mentioned that this was the first approved impregnation of uniforms in Dutch troops. Due to national legislation permission was required from the Ministry of Agriculture, which was granted a few days before departure only. Therefore it could not be applied until arrival in theatre. Specific immunisations included meningitis A+C and yellow fever. When necessary booster vaccinations were administered for diphtheria, tetanus and poliomyelitis (DTP), typhoid, hepatitis A and B. First choice for malaria chemoprophylaxis consisted of weekly mefloquine (250 mg), alternatively daily doxycycline (100 mg) was offered for those who had experienced (possible) mefloquine adverse events during previous deployments. After return the troops received a personal medical check-up, including testing on tuberculosis.

Living conditions

Troops stayed in tents on five campsite locations divided over the central part of Southern Eritrea (4) and Northern Ethiopia (1). The altitude of the campsites varied between 1500-2200 meters above sea level. Much attention was paid to hygiene such as prefab showers, toilets and dining facilities. During deployment food and bottled potable water were required from approved distributors. All had a 2-week midterm rest and recreation leave which was spent in The Netherlands. One or two supervised day-breaks were organised locally.

Medical support

The medical support plan was based on a paper risk assessment and a pre-deployment fact finding mission in Eritrea. Medical facilities in theatre included first line sickbays (BME) and one centrally located second echelon field hospital (FDS) offering surgical, X-ray, ward and additional laboratory facilities. Medical personnel received specific training in tropical medicine. In addition to ground ambulance support dedicated helicopter capacity for medical evacuation was available in theatre. The Royal Netherlands Airforce provided out of theatre medical evacuation. A preventive medicine team performed regular hygienic controls and occupational health expertise was available on call in The Netherlands.

Health surveillance

Data collection was performed in several ways. During deployment all consultations, diagnoses and lost working days were entered into the international EPINATO system. This widely used basic database divides

all consultations over some 24 diagnostic groups. Data were collected on all campsite locations on a weekly base. After initial scanning on site by the chief medical officer, monthly figures were sent to the Netherlands for further examination. Due to restrictions of EPINATO some outcomes, such as non-availability and consultation rate had to be derived. However in addition to EPINATO, the national naval database on disease and injury registration (GIFKOM) was used. This "on line" system provided more accurate information, but for this study it was used as control system only. Finally the personnel office (J-1) provided valuable information on the daily number of soldiers on site and repatriation figures.

About 14 days after return to the Netherlands all participants had an individual medical check up by a doctor. Before this examination troops were asked to fill in a post deployment questionnaire containing questions on their health and compliance to preventive medical policies.

Statistical analysis

This study is restricted to the registration data only. The incidences are expressed per 1,000 person-days. Lost men days are expressed as a percentage of the total observation days. Post deployment questionnaires were analysed with SPSS.

RESULTS

Health surveillance

The mean number of personnel in Eritrea and Ethiopia was 915 (range 759 – 1089). A total of 148,841 person-days in theatre were registered. The mean non-availability due to medical reasons was 0.41% of the total population (range 0.22 –0.64%). The mean overall consultation incidence was 18.6 (range 13.8 – 21). Additionally the consultation rates, indicating the percentage of the study population that consulted the medical system per month, were derived. From the GIFKOM-database we learned that there was an average of 1.2 consultations per diagnosis. Based on the EPINATO results this indicated that some 39% of the study population visited the local sickbays at least once every month.

In EPINATO all consultations were divided into several diagnostic groups. By the end of the mission the top 3 consisted out of "Orthopaedics & Injuries" (24.9%), "Dermatology" (22.3%) and "Miscellaneous" (20.6%). During the deployment one case of malaria falciparum was diagnosed. After return in The Netherlands another case of *p.f.* malaria was diagnosed (5 days after return) and up to one year after return 5 cases of *plasmodium vivax* malaria could be attributed to the participation in UNMEE.

Finally a total number of 25 repatriations to The Netherlands for medical reasons were registered. The main reasons for repatriation were 12 "orthopaedics & Injuries (48%) and 7 "mental disorders" (28%). Seven out of the 12 repatriations for orthopaedics & injuries returned to Eritrea before the end of the mission. None of the mental disorders returned to Africa, however by the end of the mission 6 were serving again on a different location.

Post deployment questionnaire

From the 982 troops that returned to the Netherlands 776 (755 males and 21 females) could be seen for a post deployment medical check up 14 days after return in the Marine Barracks in Doorn, The Netherlands. Before troops were seen by a doctor on an individual base they filled in a post deployment questionnaire, which was

used as guideline for the medical check-up. In this questionnaire 76.7 % indicated consistent use of malaria chemoprophylaxis, 15.4 % reported inconsistent use, while 2.8 % never used at all. Concerning bed nets: 31.6 % always used a bed net, 18.2 % mostly. 24.4 % sometimes and 25.8 % indicated non use. About 30% of the bed nets and 10 % of the uniforms proved to be impregnated. Consistent use of DEET was reported by 1.5%, inconsistent use by 36.9% and 61.6% indicated no use at all.

DISCUSSION

The main outcomes of this study show that the collective non-availability remained very low and no serious diseases occurred during deployment. The type of study and the restrictions in registration do not allow clear explanations, however we believe that the adequate individual and collective preparations, such as infrastructure and hygienic and preventive medical measures contributed to this result. Furthermore the altitude of the campsites must have minimised the (nocturnal) risk for tropical infections. From anecdotal reports we learned that troops were more concerned about their cold weather sleeping bags than the consistent use of impregnated bed nets. This may well have influenced the poor results on the use of personal protective measures.

In this respect it is remarkable that we found such a high score on consistent malaria chemoprophylaxis. Based on previous experiences with reluctance to mefloquine we decided to offer troops different options. Mefloquine was the first option but doxycycline could be used as an alternative. Much attention was paid to information on the malaria chemoprophylaxis and this could well explain why the majority chose for mefloquine.

The registration findings over the different diagnostic groups were in line with other studies on health surveillance during peace support operations. Most musculoskeletal disorders were related to working conditions and physical training. Because of strict traffic rules the number of road accidents remained very low. The daytime tropical conditions explained the high number of skin disorders. The only tropical related infections we experienced were individual cases of travellers diarrhoea. The number of dental complaints remained very low. We attribute this to a consistent preventive dental policy in the Dutch Navy.

The finding on poor compliance to personal protective measures are reason for concern. We already mentioned the altitude of the campsites as one of the possible explanations for this result. Furthermore this was the first mission which allowed the impregnation of bed nets and uniforms. The strict legal rules and late permission must have influenced the low scores.

In conclusion, despite numerous health risks, this part of Africa remained very benign to the Dutch troops. Health monitoring will remain a integral part of the medical support plan for military missions, however the registration methods need further refinement.

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14. Abstract

Medical Surveillance of military personnel will provide valuable information, not only to medical doctors but to commanders and to policy makers as well. The challenge is to develop a future oriented system-of-systems approach and to ensure user friendliness in order to guarantee adequate quality of data input.

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