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14. ABSTRACT

A population-based, cohort study was conducted to determine the importance of Gulf War deployment to Southwest Asia, from 1 August 1990 to 31 December 1991, in explaining neurologic mortality and peripheral nerve disease among United States military working dogs. The study cohort consisted of 2,123 military working dogs that were eligible to deploy to the Gulf War and died between 4 September 1990 and 30 June 2001 with complete medical records maintained at the Department of Defense Military Working Dog Training Center and Veterinary Services, Lackland AFB, San Antonio, TX. Within this Gulf War cohort was a prospectively followed cohort of 651 dogs; 347 of these dogs had complete peripheral nerve histopathologic diagnostic records. Descriptive analysis of the study variables defined neurologic mortality incidence for the Gulf War cohort at 1.90 cases per 1,000 dog-months. Rates among dogs assigned to the United States, overseas, and Southwest Asia were 1.83, 1.91, and 2.44 cases per 1,000 dog-months, respectively. Analysis of other exposures showed highest neurologic mortality among dogs assigned to Southwest Asia countries other than Saudi Arabia, dogs arriving before or departing after the war, dogs that arrived before and departed after the war, and dogs that spent more than 176 days in Southwest Asia. Peripheral nerve disease incidence was 3.69 cases per 1,000 dog-months for the prospective cohort. The rate for dogs assigned to the United States was higher than for those overseas or in Southwest Asia (3.86, 3.53, and 3.21 cases per 1,000 dog-months, respectively). Using survival analysis, adjusted neurologic mortality and peripheral nerve disease rates were similar between the United States, overseas, and Southwest Asia assignment locations. An increasing trend of neurologic mortality was evident with increased time spent in Southwest Asia. Results from this study suggest that there was not a relationship between Southwest Asia deployment during the Gulf War and neurologic mortality or peripheral nerve disease. This study is consistent with previous human Gulf War neurologic studies. The military working dog remains a viable sentinel model for measuring outcomes of unique military exposures. This study and available data sets can form the basis for future studies.

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ABSTRACT

Title of Thesis: Dogs	Risk of Peripheral Nerve Disease in Military Working Deployed in Operations Desert Shield/Storm
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RISK OF PERIPHERAL NERVE DISEASE IN MILITARY WORKING DOGS DEPLOYED IN OPERATIONS DESERT SHIELD/STORM

By

Kelly G. Vest, DVM, MPH

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DEDICATION

To my children that bring joy into my life:

Michael for his light heartedness to see the things that are good in times that are bad; Amy for her determination to overcome physical obstacles is an inspiration; David for his gift of kindness and longsuffering and always do what is right; he is an example to me; Aaron for his sense of adventure and excitement to do anything he sets out to do; To Leah for her sweet smile that brightens my every day; And to Matthew for his wonderful hugs that brings happiness to my soul; To my wonderful wife, Jeanine, who has given me her love, support, and understanding, enduring with me through these many years of work and school adventures.

I love you all.

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CHAPTER 1: INTRODUCTION

Background and Significance

Beginning in August 1990, the United States (U.S.) deployed nearly 700,000 troops to Southwest Asia (SWA) in response to Iraq's invasion of Kuwait. This defensive build up of military personnel, the period known as Operation Desert Shield, continued until mid-January 1991 when the U.S. phased into the offensive operation known as Operation Desert Storm. The offensive period began with an active air war lasting 39 days and ended with a four-day ground war in March 1991 when Kuwaiti leaders were returned control of their nation. The final military phase involved redeployment of forces back to the U.S. with the majority of forces arriving home by July 1991. By December 1991, new U.S. bases and areas of operation were negotiated, designated, and established for long-term regional sustainment [1, 2].

Soon after the war, numerous Gulf War (GW) veterans presented with varying health complaints, some of which were non-specific in nature, often hard to diagnose, quantify, and connect to a source or cause [3]. These physical symptoms involve multiple organ systems, include fatigue, headache, joint pain, sleep disturbances, memory problems, shortness of breath, impaired concentration, malaise and other various signs [1]. This set of symptoms, frequently labeled the GW "syndrome", has beset the U.S. Armed Forces for over a decade. These veterans voiced concerns about the possible connection between their health and their deployment exposures during the war [3-6].

The U.S. Government, under the guidance of President Clinton, established different programs through the Department of Defense (DoD), Department of Veteran Affairs (VA),

and Department of Health and Human Services (DHHS) for the evaluation of GW veteran's health status and directed federal researchers to "leave no stone unturned" [1, 5-8]. The efforts included various work groups, panels, databases, and health registries that would allow flexibility and opportunity to perform many epidemiologic studies between the health outcomes and the potential occupational exposures of U.S. Armed Forces personnel in a theater of war.

President Clinton reiterated in the Presidential Review Directive-5 that his emphasis was on an independent, open, and comprehensive examination of health concerns related to GW service [9]. This document focused on the extent of assessing what can be learned from the GW population and applying these lessons learned to improve, oversee, and prevent poor health outcomes during future deployments. The President re-emphasized the importance of scientific research and delegated responsibilities to the DoD, VA, and DHHS.

Since 1991, hundreds of studies have been published concerning the illnesses of GW veterans. The results have not provided support for a specific syndrome or new condition connected with the deployment of troops to SWA [1]. The Final Report from the Presidential Advisory Committee on Gulf War Veterans' Illnesses, states that GW veterans have experienced no excess mortality from natural causes during or after the war. Most of the symptoms and illnesses have been explained, but some have not. Furthermore, many studies suggest study uncertainty associated with a perceived stigmatization of psychosomatic illness for some veterans and personal perceptions of illness among other veterans. Some of this bias has been explained, but most has not. Stress also appears to be considered an important factor in the demonstration of many symptoms [10, 11].

Many of these symptoms could be associated to neurologic disease, such as muscle and joint pain, numbness and weakness in the limbs, and loss of coordination and reflexes. Moreover, exposure to pesticides and volatile organic compounds used during the GW could increase the risk of neurologic disease [1, 5, 12-14].

Use of sentinel animals provides opportunities to look at many similar acute and chronic conditions quickly and in a shorter period of time compared to humans [15]. Some studies have suggested using animals to evaluate environmental contamination of neurologic agents [16, 17]. This study used the military working dog (MWD) population to further examine neurologic disease and GW exposure.

The MWD population can demonstrate comparable symptoms and outcomes found in man. Historically, the canine model has proved to be valid for many pathologic conditions in humans, including nerve and soft tissue problems [15, 18]. The MWD spend much of their life in a similar environment as soldiers, airmen, sailors, and marines. They are exposed to the same environmental hazards as their handlers and other Service members. Military working dogs may have greater exposure and absorption associated with the direct contact with ground and soil surfaces and dust, including increased ingestion of contaminants on their fur due to grooming. Such a population of individuals should not be overlooked in the exploration and development of relationships between SWA deployment and neurologic disease.

Some studies have shown evidence that domestic animals respond to certain environmental situations with what might be considered a stress response, both in immune physiology and behavior[15]. Yet, observation of the animal's responses may differ from the observation and assessment of the human's responses that may be biased through self-

reports. Therefore, the benefits of using MWD include reducing many of the human psychological and behavior factors that may bias reports of clinical symptoms and health conditions. These behavioral factors that may bias self-reporting include the effects of the personal perceptions of illness and exposures, the psychological impact of stress, and self-imposed behavior exposures like drugs, alcohol, and smoking.

Pilot Study

A pilot retrospective observational survival analysis had been performed evaluating the survival of GW-era MWD with neurologic, neurologic-related, and neoplastic mortality outcomes using a sample of 1368 archived GW-era MWD mortality records. The final models were evaluated with Cox regression and adjusted rate ratios (RR) were determined. The rate of dying from a neurologic cause for a GW veteran dog was increased 2.1 times (RR=2.10; 1.29, 3.49) the non-deployed dog rate, adjusting for breed and gender. Furthermore, breed had significant impact on mortality. German Shepherd Dogs (GSD) died from neurologic conditions at a significantly higher rate than Belgian Shepherd Dogs (BSD) (RR=2.06; $p<0.001$; 1.43, 2.96) even after adjusting for deployment status and gender [19].

Literature Review

Government and Policy Document Review

The U.S. Government has been directly involved in GW medical research and in assisting those who have reported an unspecified illness since their deployment to the GW. Soon after the war, numerous GW veterans presented with varying health complaints, some

of which were difficult to diagnose or ill-defined illnesses. Veterans voiced concerns about the possible connection between their health and GW deployment exposures [3-6]. The U.S. Government established different programs through the DoD, VA, and DHHS for the evaluation of GW veterans and their subsequent health status [1, 5, 6, 8]. These included various work groups, panels, databases, and health registries that would allow flexibility and opportunity to perform many epidemiologic studies between the health outcomes and the occupational exposures of U.S. Armed Forces personnel in a theater of war.

The Presidential Advisory Committee on Gulf War Veterans' Illnesses, in its final report on the nature of the GW illness reported on analyses of the veterans evaluated through the VA GW Registry and the DoD Comprehensive Clinical Evaluation Program (CCEP) [5, 6, 10]. The morbidity frequencies found within these two programs were similar. The top three diagnostic categories reported for both the CCEP and the VA GW Registry include psychological conditions, musculoskeletal system diseases, and symptoms, signs and ill-defined conditions. Neurologic disease was diagnosed at a frequency of 5.7 percent and 8.3 percent for the CCEP and the VA Registry, respectively. The Committee concluded that GW veterans have no excess mortality from natural causes but there was an increase in external mortality causes due to motor vehicle accidents. The Committee suspected that many veterans have illnesses that are likely connected with their Service in the Gulf, but the extent of the illness is unknown. Additionally, they reported that stress related disorders should not be overlooked, although stigmatization associated with a psychological label for their illness interferes with veterans seeking care. Recommendations included focusing on the causes and the prevention strategies of musculoskeletal conditions, stress-related disorders, and stigmatization.

Risk factors and environmental exposures were reviewed. Although some veterans clearly have service-connected illnesses, the Presidential Advisory Committee felt that current scientific evidence does not support a causal link between the symptoms and illnesses reported by GW veterans and exposures to the following environmental factors while in SWA: pesticides, chemical warfare agents, biological warfare agents, vaccines, pyridostigmine bromide (PB), infectious diseases, depleted uranium, oil-well fires and smoke, and petroleum products. The pesticides used during the GW were in five major categories: organophosphates, methyl carbamates, organochlorines, pyrethroid pesticides, and *n,n*-diethyl-m-toluamide (DEET) [3, 10, 14]. The Committee further recommended that long-term mortality studies of GW veterans continue. The conclusions emphasized that the physiologic effects of stress and stress-related disorders should not be overlooked, as stress is likely to be an important contributing factor to the broad range of physiological and psychological illnesses being reported by GW veterans [10].

The DoD CCEP began as an ongoing study of 18,598 GW veterans [6]. Initial findings show that in comparison with U.S. general population, the reported types of symptoms are not unique and are similar in nature to those seen in other groups of patients. The wide variety of symptoms involves multiple organ systems with no consistent clinical pattern. When compared to other reports that also exhibit unexplained diagnoses, the CCEP participants have similar outcomes of unexplained diagnosis with these types of symptoms. The majority of diagnoses fall in the four categories of 'psychological conditions,' 'symptoms, signs, ill-defined conditions', 'musculoskeletal and connective tissue diseases,' and 'healthy.' A report of the CCEP findings concluded that GW veterans have real problems, symptoms, and illnesses, but generally they are able to function in their jobs.

There was no clinical evidence to suggest that there is an unknown, serious illness or syndrome among GW veterans.

The VA initiated a GW Health Registry that includes 52,835 veterans [5]. The Registry enrollees are similar in ethnicity, but are older and exhibit a higher proportion of females than the GW cohort (10.4% vs 7.2%, respectively). The VA Registry had an overrepresentation of reservists than active duty (39.1% vs 16.7%, respectively) and U.S. Army (USA) personnel. The most frequently diagnosed conditions were diseases of musculoskeletal and connective tissue. There were approximately 14,000 individuals with undiagnosed illness. Medical conditions reported by GW veterans are similar whether individuals were assigned to an active unit or a reserve unit and whether individuals served in ground units or on ships. Generally, the frequency of reported symptoms increased over time, whereas the proportion of individuals with physician-diagnosed illnesses remained fairly constant. The report conclude that registry participants report a wide variety of health problems that appear to be different from what has been seen in other armed conflicts, yet no new illness or single syndrome has been identified [5].

Alternatively, Hyams et al. expressed a different view in their study of the medical outcomes of U.S. participants in wars from the U.S. Civil War to the GW [20]. This study reports that war syndromes have been associated with wars at least since the Civil War. These war syndromes exhibit similar symptoms with no specific disease, but they all have the same factors involved: a unique population that was intensely scrutinized after experiencing an exceptional, life-threatening set of exposures. However, because the location and nature of each war varies, they feel it will be extremely difficult to answer all health questions and to predict every health risk of war.

A similar study examining post-war syndromes among British veterans including all major military conflicts from the Boer Wars to the GW reported similar results [21]. Syndromes have been described after every conflict, but one particular syndrome has not been found to be common over all. Three syndrome types appear to be associated with the nature of the war. The variations between these three syndromes were determined by the nature of the combat environment, the medical knowledge, and the health beliefs and fears of the veterans and physicians present during that particular time in history. Jones et al. feel that post-war syndromes will always be a part of war; the aftermath of the syndromes will continue to express themselves in different ways depending on how symptoms and experiences are perceived by the veteran and interpreted by the physician.

Of those who deployed to SWA, there were a large number of military reservists (~17%); many of those with ill-defined illnesses were reservists (~39%) [5]. Many of the illnesses have been diagnosed with few cases categorized as symptoms, signs and ill-defined conditions. However, even with few cases categorized as ill-defined conditions, this does not provide a reasonable answer to those veterans who feel they were affected in some way by the war and are requesting subsequent medical coverage and compensation for their Service-related illnesses [22].

In addition to questions about their health, these individuals were concerned about not having appropriate health care coverage, associated with their present military status (reserve, veteran, or retired), for the ailments that overwhelm them physically, emotionally, and financially. Many military and VA health policies and regulations have been reconsidered and adapted to provide improved health care access for those veterans who perceive their condition to be connected to the war [4, 22, 23]. In December 2001, President

Bush signed into law the Veterans Education and Benefits Expansion Act of 2001. This Act provided increased medical compensation and coverage until 2011 for GW veterans exhibiting one or more of 13 medical signs and symptoms [24]. These symptoms are fatigue, unexplained rashes or other dermatological signs or symptoms, headache, muscle pain, joint pain, neurologic signs or symptoms, neuropsychological signs or symptoms, signs or symptoms involving the respiratory system (upper and lower), sleep disturbances, gastrointestinal signs or symptoms, cardiovascular signs or symptoms, abnormal weight loss, and menstrual disorders.

Disease and Injury among Gulf War Veterans

General

Studies of GW veterans have looked at different aspects of their health and many different types of exposures. A 1998 cross-sectional, population-based, survey looked at 1,548 veterans of the GW and 482 non-GW veterans from Kansas [2]. This study defined the GW "illness" as any illness with symptoms similar to, and that exceeds, those of non-GW veterans. It looked at deployment location and four different deployment periods within which the veterans would have arrived in and left from the theater: August 1990 to January 1991, January 1991 to March 1991, January 1991 to April/May 1991, January 1991 to June/July 1991. The results of this study showed that the prevalence of GW illness was reported lowest among those serving on ship and highest among those in Iraq and/or Kuwait. Illness was least prevalent among those who departed the region prior to January 1991, and most prevalent among those who departed in June/July 1991. Observed patterns suggest that excess morbidity among GW veterans is associated with characteristics of their wartime

Service, with self-reported receipt of vaccines, and with being female. No association between illness and age, income, education, rank, component, or branch of Service was found.

Another study also considered the time periods of veterans service: pre-war, during war, post-war, and combined periods [13]. This study was a clinical case-control study, involving GW veterans from the northwest region of the U.S. Unexplained symptoms were categorized into three groups: musculoskeletal, cognitive/psychological, and fatigue. The results showed no statistical differences in the proportion of cases within the three complaint groups found in each Service period. However, similar to Steele's results, they reported increased rates for all symptoms of the GW veterans who served in the post-war period.

Using a retrospective cohort approach, Gray et al. compared different time periods and the association with hospitalization experiences [25]. In this approach, however, the time periods were set to look at hospitalization events prior to August 1990 and three periods after the war starting in August 1991 through December 1991, the 1992 year, and January to September 1993 period. They concluded that hospitalization experiences of GW veterans due to unexplained illnesses were not higher than that of Service members who were on active duty but had not deployed to SWA.

Instead of morbidity, Kang et al. performed a 1996 retrospective cohort study of the mortality rate of GW veterans [26]. They reported that the mortality rates showed a significant increase of deaths among GW veterans over non-deployed veterans associated with auto accidents. The overall mortality rates of both veteran groups were significantly lower than that of the general population of the U.S.

Seven years later, Kang et al. followed up on the GW veterans mortality rates and performed a survival analysis [27]. The GW veterans' and non-GW veterans' mortality rates remained less than half that expected in the standard population, yet they maintained a higher risk of mortality associated with motor vehicle accidents than their civilian counterparts. It was noticed, that over time, the GW veterans' protective rates of all causes of mortality disappeared and the GW veterans became similar to the non-GW veterans.

As an editorial on the studies produced by Kang and Gray, Campion focused on the evidence that no new illness had been identified from looking at morbidity and mortality information [28]. He makes the comment that despite the lack of a clearly defined disease entity, the government now offers compensation to any GW veterans with long-term disability due to unexplained symptoms that began during or after the war. In a different commentary, Steele, referring to the Kang studies, remarks that GW veterans report unexplained symptoms at higher rates than non-GW veterans comparison groups, but they don't experience increased rates of disease related mortality [29]. He continues by stating that the GW veterans have not been unduly affected by adverse health outcomes (mortality) and the health problems are not of the type that generally results in premature death or hospitalization, yet, they have consistently been shown to be more symptomatic and debilitated. Steele concludes with his support of the use of epidemiological methods to better define the GW illness case.

Similar results have been reported among British GW veterans in a 1999 cross-sectional study of those who deployed to the GW or to Bosnia, and those service members that did not deploy [30]. No unique syndrome was identified, although the frequency of symptom reporting was higher in the GW cohort compared to the others.

A 2001 case-control study looked at the association of posttraumatic stress symptomatology (PTSS) and GW veteran's self-reported and medically undiagnosed illness [11]. Ford et al. found that PTSS and somatic complaints were independently associated with case status, along with war zone trauma and depression. They suggested that PTSS be considered a potential contributor to physical health problems experienced by GW veterans supporting the recommendation of the Presidential Advisory Committee on Gulf War Veterans' Illnesses.

Another author suggested that the overlapping multi-system, multi-organ illnesses are evidence of an autoimmune disorder. Internal physiologic and chemical changes in the body associated with the combined exposure of PB, adrenaline, biological stressors and other chemical exposures (i.e. DEET) simply result in an unclassified autoimmune disorder [31].

To summarize what is known and has been found in the hundreds of GW studies, a comprehensive review of most available literature and studies through 1997 that address GW health issues was published in 1998 [1]. The following insight and conclusions were made: most GW veterans have diagnosable and treatable conditions; overall mortality and hospitalization of GW veterans appears favorable compared to the general population; it is likely that GW experiences have been a contributing or aggravating factor in the development of some veterans' illnesses; and investigations to date have been unable to identify a direct causal link between the symptoms and illnesses and certain occupational and environmental exposures.

Gulf War Illness and Neurologic Studies in Humans

Neurologic symptoms and disease have been a focus of study among the GW veterans. Many of the environmental exposures that could or did occur in SWA could have a

neurologic toxicity. It is well known that many chemical exposures cause acute neurologic symptoms and signs, yet in the case of the GW veteran the self-reported symptoms suggest a chronic nature to the illnesses. The issue at hand is whether long-term chronic conditions are associated with the short-term and/or low-level exposure to these chemicals and environmental contaminants during the deployment time period.

The nonspecific nature of the symptoms does not point to a specific agent so investigators have studied various environmental exposures that have effects on the neuromuscular system, including chemical warfare agents, medications, vaccines, and pesticides. Among humans, a 1999 VA study reported that neuromuscular complaints were cited among 16.8 percent of 52, 835 GW Health Registry veterans [5]. Several other GW veteran studies focusing on peripheral nerve disease (PND) outcomes reported normal results and no evidence of any specific pattern to the patient's complaints and symptoms [32-34]. Nevertheless, one of these studies found small but significant differences in large fiber peripheral nerve function and thinly myelinated small fiber function [35], and another found a significant relationship between self-reported neurological symptoms and self-reported exposures to neurotoxic agents [12].

A 1995 retrospective study, using the CCEP registry information, evaluated the neurologic disease outcomes of 65 patients [33]. Newmark et al. concluded that there was no common pattern of nervous system dysfunction in this sample of veterans, nor was a new syndrome identified. The most common complaint was headache. There was a high frequency of sleep disturbance and approximately 50.0 percent had an abnormal neurologic exam. The authors concluded that sleep disturbances may be under diagnosed.

Other studies observed similar inconclusive results. One 1997 observational prospective study of 20 GW veterans examined neuromuscular complaints, nerve biopsies, and nerve conduction studies [32]. They concluded that there was no evidence of a neuromuscular disorder and that any exposures to environmental toxins in SWA were not responsible for the patients' symptoms. More recently, a 2001 cross-sectional study of 176 Puerto Rican GW veterans demonstrated no definite generalized neuropathic pattern [34].

Three studies suggest that neurologic disease outcomes are related to participation in the GW. One of these studies is a pilot case/control study of GW veterans from the United Kingdom [35]. Significantly abnormal results were identified in peripheral nerve function, specifically sural nerve latency and median nerve potential. In particular, small, but significant differences were found in large fiber peripheral nerve function and thinly myelinated small fiber function. The other two studies were performed among the GW veterans of a Reserve Naval Mobile Construction Battalion [12, 36]. One study was a cross-sectional survey[12] and the other was a case-control study[36]. The latter study concluded that among these GW veterans, the outcomes appeared to represent variants of a generalized injury to the nervous system; the cross-sectional survey found a significant relationship between the self-reported neurological symptoms and self-reported exposures to neurotoxic agents such as the use of flea collars, insect repellants, and the use of PB.

Peripheral Nerve Disease Epidemiology in Humans

The worldwide incidence for peripheral neuropathy in humans has been reported at 1 per 25,000 persons per year with a worldwide prevalence ranging from 4.7 to 36 per 100,000 [37]. The peripheral neuropathy incidence in the UK was reported at 118 cases per 100,000

persons per year [38]. Hereditary motor and sensory neuropathy, also known as Charcot-Marie-Tooth (CMT) disease, incidence has been reported at 1 per 2,500 persons per year in the United States [39]. Many studies have shown a pattern of nerve degeneration in healthy individuals as they age [40].

Sentinel Animal Studies

Animals have been used throughout time as sentinels for events and environmental exposures. Canaries as indicators of coal gas (carbon monoxide) exposure in coalmines are well-known examples of the use of animals to assist in determining human health risk. O'Brien et al. wrote, "Sentinels perform the function of calling attention to the interrelationship between human health and animal health with respect to the environment [18]."

Animals have been used in research and in investigating the risks of human exposure to environmental agents, both toxic and infectious. Prior to the diagnosis of human disease, neurologic symptoms associated with mercury toxicity were first observed in cats in Minimata Bay, Japan in the 1950s. Animals also have been indicators for environmental contaminants and toxins such as polychlorinated biphenyls, insecticides, chlorinated naphthalenes, aflatoxin, and polybrominated biphenyls [15]. Dogs have been sentinels in cases of toxicity due to lead, mercury, tetrachlorodibenzo-*p*-dioxin, and polychlorinated biphenyls [18].

The National Research Council's report on the use of animals as sentinels, lists some of the attributes that make an animal a good sentinel. The animal should have a home range

territory that overlaps the area to be monitored. A sentinel species should be easily enumerated and captured, and have a sufficient population size and density to permit enumeration. And finally, there should have a measurable response to the agent or class of agents in question [15].

Some of the benefits of using animal sentinels include: providing information on exposure, identifying relative risks, and gathering medical data that may be relevant to similar human diseases. Animals develop outcomes faster in chronological time than humans. Animal sentinels do not share the biasing or confounding effect of human behavior and self-report. In some cases, animal sentinels can reduce uncertainties by providing data on animals exposed in parallel to the humans whose risks are to be determined [15, 18].

Along with benefits, there are limitations to sentinel systems. For the most part with any sentinel system, there is the difficulty of standardizing data and extrapolating conclusions to humans. Cross-species differences and similarities in biology, behavior, and other significant characteristics have not been studied in sufficient depth to adjust and validate the data for utilization across the board. An example is a 1983 study of family pets in Missouri after environmental exposure to 2,3,7,8: tetrachlorodibenzodioxin (TCDD) [41]. The results suggested that pets in contaminated areas might have been at greater health risk. Extrapolation to the human population was thought to be inappropriate, however, due to a small sample size, recall bias of the owners, and unconfirmed medical outcomes of the animals, the authors cautioned against use of owner-reported data.

Even without these limitations it is unlikely that sentinel animal data would be only determining factor for human health assessments. Sentinel animal health information may be

used best as an early warning for unseen risks that may lead to further studies, provide evidence for a risk assessment, or suggest support for a cause and effect relationship [42].

An example of a project using sentinel animals in assessing human health risk was a case-control study of pet dogs with mesothelioma matched with controls and with their respective owners comparing the association of asbestos related exposures of the owners in occupational and hobby settings with cancer outcome in the animals [43]. Cancer in the dogs was significantly associated with asbestos related occupation and hobby exposure of their owners (OR=8.0, 95% CI 1.4, 10.6). There also was an association with the exposure to pesticides that contained chrysotile fibers (OR=11.0, 95% CI 1.5, 82.1).

The MWD has been utilized in sentinel type studies. A 1968 prospective study compared the serological response to natural arboviral exposures in Thailand and Vietnam between the MWD and their handler [44]. The study reported a coupled, agreeable response in serum titers for chikungunya fever, and Japanese-B encephalitis arboviruses between the MWD (2.6% and 41%, respectively) and their handlers (3.1% and 34%, respectively) for both viruses.

A 1975 prospective report of 60 MWD in southern Florida surveyed them for serum titers for the Everglades virus (Venezuelan equine encephalitis) and Saint Louis encephalitis. Ten percent of the MWD either initially tested positive or sero-converted to Everglades virus [45]. An additional 5.0 percent of the MWD sero-converted against Saint Louis encephalitis.

After the Vietnam War, two MWD studies evaluated the testicular seminoma outcomes of MWD that served in the Vietnam theater of operations [46, 47]. Each study demonstrated a two-fold difference between Vietnam veteran MWD and continental U.S. (CONUS) based MWD who died between the years 1968-1978. The 1995 study showed that

military service in I Corps area of operation was associated with a significant risk of testicular seminoma (OR=3.1, 95% CI 1.9, 4.9). To complement these studies, a 1991 human case-control study among Vietnam veterans examined their risk of testicular cancer [48]. A two-fold increased risk for testicular cancer was observed in Vietnam veterans.

Sentinel animals already have been used for a GW study. A 1994 cross-sectional study evaluated pathologic outcomes in 26 feral cats exposed to Kuwait oil fire smoke [49]. Moeller et al. reported that the histopathologic outcomes in the cats showed that the inhalation of air contaminated with smoke from the oil fires had little or no long-term affect on the animals examined.

Canine Breed Characteristics

Military working dogs are not a homogeneous population, so breed-specific health differences may be important. In the veterinary literature, there are few canine breed population studies that define the prevalence and incidence of morbidity or mortality by breed and/or sex. Most of the literature is laboratory-based studies or individual anecdotal clinical and/or case reports.

Seven MWD studies do provide some observed demographic, morbidity, and mortality distributions. The MWD population historically has primarily been composed of working breed canines, particularly those found within the German Shepherd Dog (GSD) breed; beginning in the mid-1980s the Belgian Shepherd Dog (BSD) breed became popular. Other breeds have been used for specific purposes, but the Shepherd dog breeds have the disposition looked for in a military-style animal and tend to be the most popular. In his book,

Medical and Genetic aspects of Purebred Dogs, Ross Clark describes the recognized problems of the BSD and the GSD [50]. Neuromuscular related problems in the BSD include hip dysplasia, epilepsy, and reduced levels of thyroid hormone. Those found in the GSD are hip dysplasia and progressive posterior paresis. All conditions may mimic or involve direct or indirect neurologic deficits. Both breeds have an average age at death of 12 years.

Five studies give breed-specific characteristics and disease incidence rates for the GSD in the general population. A 2001 retrospective study used necropsy reports of 1,206 dogs to describe the causes of death in 5 breeds [51]. For GSD, 63.7 percent of all causes of death were attributed to non-neoplastic causes. Under non-neoplastic causes, specific information on lameness or neurologic attributed mortality was not reported; it is likely these were included under the 'Other' or 'Open' cause subcategories in which there was a combined prevalence level of 16.0 percent. GSD mean age at death was 6.2 years for non-neoplastic, 7.3 years for all causes, and 9.1 years for neoplastic deaths.

A retrospective study in 1982, looked at 2,002 necropsy results over a 15 year period from an animal hospital in Boston [52]. Fifty-six breeds were examined. The mean age at death for GSD (n=207) was 5.4 ± 3.9 years. Particular information on specific mortality causes was lacking. Generally, neutered or spayed animals tended to live longer than intact male or female dogs. The short-lived dogs died of a wide variety of diseases often appropriate for age, but not old age diseases; whereas, long-lived breeds died of diseases also appropriate for age, but particularly those associated with old age, such as cancer.

Three related studies are probably the most involved population studies of canine breeds and specific mortality causes that have been published [53-55]. The authors used the

database of a Swedish companion animal insurance company and studied the morbidity/mortality claims of over 200,000 dogs. Age patterns of mortality were explored among the various breeds of dogs [54]. For GSD, the risk of survival at five years was 83.0 percent and at ten years was 53.0 percent. The authors recommended that for studies of mortality in dogs, the use of the actual age with multivariable analysis is a better method than ignoring age as a confounder. In a retrospective cross-sectional study, they found that the overall incidence of reported neurologic mortality was 0.2 percent among all dogs with at least one clinical visit out of all dogs at risk [53]. Neurologic mortality was reported in 0.9 percent of all insurance claims among all female dogs and 1.5 percent in all male dogs. German Shepherd Dogs most commonly had morbidity attributed to the skin (31.7%), gastrointestinal (19.0%), genital (17.9%), unspecified (16.2%, unspecified lameness and lethargy), and respiratory (7.8%) systems. In a retrospective cross-sectional study, GSD demonstrated a cause specific mortality for locomotor system disorders of 21.4 percent of all deaths in GSD [55].

Military Working Dog Studies

The majority of MWD studies in the literature are descriptions of the Vietnam-era MWD, primarily studies of the symptoms and treatment of arboviral, tick-borne, and other infectious diseases. Seven MWD studies, four from the Vietnam era, one from Europe, and two recent studies that incorporate some of the GW MWD cohort, define trends in overall MWD morbidity and mortality.

One of the founding articles on MWD mortality causes is a 1973 retrospective study of histopathologic results of 2,500 GSD (91% males) that had served in the Vietnam theater

of operations and had died between 1964 and 1971 [56]. Of the total MWD records reviewed, 46.0 percent did not have a causative agent identified, but did have a diagnosed pathologic lesion. Of the MWD that did not have an identifying etiologic diagnosis, 5.6 percent of these dogs had primary nervous system histopathologic lesions. The seminoma studies were conducted with a subset of this population [46, 47].

Hayes et al. published another study in 1994 that provided a description of the Vietnam-era MWD [57]. The mean age of those MWD that died in Vietnam was 5.7 years whereas the mean age of those that left Vietnam and died in other areas of the world was reported at 6.5 years and 9.4 years for the cohort years 1968-1973 and 1974-1980, respectively. Specified causes of deaths in these two cohorts where not discussed except for deaths caused by hostile action, gastric dilation volvulus, heat stroke, and death due to other reasons.

Two other articles described a veterinary medical record keeping system instituted in the Vietnam theater of operations and the flexibility and benefit of the system to maintain records and survey morbidity and mortality of the MWD [58, 59]. Of the USA, USN, and U.S. Marine Corps (USMC) MWD serving in Vietnam during 1970 with six months of medical reports, the majority of the morbidity outcomes were infectious and parasitic in nature. There was no mention of neurologic morbidity, but arthritis and myalgia was diagnosed in 1.0 percent of all GSD surveyed, and in 0.6 percent of the scout and 1.5 percent of the sentry MWD.

In a 1987 survey of 123 MWD (all GSD) that died or were euthanized in Europe between 1 January 1982 - 31 December 1985, approximately 40.0 percent of the MWD were euthanized for locomotion problems as a result of their inability to perform their duty [60].

Of these dogs with locomotion problems, 98.0 percent of MWD were euthanized because of chronic recurrent lameness in the rear legs. Dutton et al. described the differentiation between orthopedic and myelopathy outcomes; degenerative myelopathy is characterized by a progressive disuse of the rear legs with marked proprioceptive deficits. Mortality was attributed to degenerative myelopathy in 6.5 percent of all causes of death/euthanasia with an average age of 9.6 years.

Moore et al. reported a 2001 retrospective study on the reasons for death and euthanasia of 927 MWD that had died between 1993 and 1996 [61]. Of these MWD, 15.6 percent died with neurologic system involvement; 13.7 percent of the BSD and 19.4 percent of the GSD had died from neurologic disease related mortality. There was a significant neurologic related mortality difference between these two breeds (OR=1.72; 95% CI, 1.14, 2.57). The mean age at death of the BSD and GSD were 9.9 and 10.2 years, respectively. Moore et al., who provided a more accurate and standardized categorical system for assigning causes of death and reasons for euthanasia for MWD, concluded: "Neurologic disease, potentially accompanied by orthopedic disease, presents a diagnostic and therapeutic challenge to clinicians of working dogs."

Burkman et al. reviewed the medical records of 90 MWD that served in SWA during the GW and looked at the primary clinical illness presentations of the MWD in the theater of operations during the GW [62]. Illness and injury accounted for 51.0 percent of all visits to veterinary treatment facilities during deployment. Of 123 total clinical presentations, 1.6 percent of the presentations were diagnosed as involving the neurologic system.

Neurologic Disease Testing

Nerve Biopsy

Opinions vary in the medical field as to the necessity of nerve biopsy because other means exist to determine the extent of peripheral nerve disease (PND) through neurologic examination, nerve conduction studies, and other electrophysiologic measures [63, 64]. In the living human patient, nerve biopsy tends to be used as a last resort to determine the extent of a degenerative neuropathy. The nerve biopsy remains a gold standard to determine the extent and stage of the neurologic condition. In some cases of diagnosing and treating a neuropathy, a nerve biopsy remains a required and necessary test [38, 63-65].

This concept is not much different in the dog; the nerve biopsy is an essential test in the diagnosis of neuromuscular diseases [66]. Although biopsy is an important and necessary step in the diagnosis of a nerve problem, the histopathologic results may only reflect a lesion at a more proximal location [67]. Frequently biopsied nerves include the common peroneal nerve, the tibial nerve, and the ulnar nerve.

Aging is associated with nerve degeneration in the dog [68, 69]. Counts of single-teased myelinated nerve fibers and cross-sectional nerve preparations from normal dogs documented lesions, including axonal degeneration and segmental demyelination and remyelination, which increase by up to 9.0 percent with age in older dogs. Minimal lesions were noticed in dogs less than ten years of age.

Neurologic Examination

Peripheral nerve lesions can cause motor and sensory deficits with signs of decreased muscle tone and weak or absent spinal reflexes involving one or more limbs on the neurologic examination [70, 71]. Additional signs of lower motor neuron dysfunction are flaccid paresis, including a short-stride gait with over flexion of joints, and a decreased ability to support weight, which produces a crouched or squatting stance. In dogs, this gait may be referred to as a 'bunny hop' in the pelvic limbs.

Nerve Conduction Studies

Electrophysiological evaluation of the peripheral nerves may assist in the diagnosis of a degenerative and regenerative process for a particular nerve [72]. The motor nerve conduction velocity (measured in meters/second; m/s) may reflect the degree of myelination of the peripheral nerve. The most common nerves used are the radial nerve, ulnar nerve, tibial nerve, and peroneal nerve.

Normal canine nerve conduction velocities have been reported at 58.9 ± 1.0 m/s for the ulnar nerve and 68.9 ± 5.8 m/s for the tibial nerve [73, 74]. Another study found that normal adult nerve conduction values are reached between six to twelve months of age with a plateau range of 60 ± 10 m/s up to seven years of age [75]. Subsequently, there is a gradual decline so that by ten years of age the normal conduction velocity is reduced by 10.0 to 15.0 percent. Limb length effects on conduction velocity; as the limb increases in length the velocity slows [73].

Serological Measures

Hypothyroidism has been associated with nerve degeneration and disease [76, 77], although, a direct causal relationship has not been confirmed [67]. Normal serum thyroxine (T4) levels have been documented at 1.0 to 3.5 μg/dl for the dog. Certain breeds, including

the BSD and GSD, have lower serum T4 values than other breeds [50, 78]. Serum T4 concentrations decrease with advancing age [79].

Measuring free serum T4 (fT4) concentration by equilibrium dialysis, which is considered the gold standard, may more consistently identify a suspected case of hypothyroidism. Normal values of fT4 for the dog are 1.0 to 3.5 ng/dl [78]. To increase the diagnostic accuracy, canine thyroid-stimulating hormone (cTSH) levels are measured in combination with serum total T4 and free T4 for defining hypothyroidism in dogs. Normal cTSH values for dogs have been published at 0.01 to 0.5 ng/ml [78]. Recent studies suggest that the cTSH test be interpreted jointly with total T4 and free T4 because it is inconsistent in hypothyroid dogs when considered alone [67, 80, 81]. A low serum total T4 and fT4 level with a high cTSH is suggestive of primary hypothyroidism and normal levels of each test rule out hypothyroidism [67, 78, 80].

Decreased acetylcholinesterase (AChE) levels are one indication of potential exposure to organophosphates or other agents that bind to AChE and prevent break down of acetylcholine at the neuromuscular junction [82, 83]. Normal blood levels in the dog are 1.2 to 3.0 IU/ml based on detectable plasma butyrylcholinesterase [83]. The normal level of AChE in healthy dogs has shown to decrease with age [84] and may decrease with routine use of insecticides [85].

Neurologic Studies in Dogs

Few studies in the veterinary literature define neurologic outcomes in GSD let alone in the BSD. Among other dog breeds, only individual case reports are found. It is not possible to generalize from one breed to the other. Chronic progressive canine peripheral

neuropathy is one of the most difficult conditions to recognize in the dog, as is the identification of a specific etiology [60, 61, 67]. The recognized etiologies include endocrinopathies, neoplasia, toxins and drugs, infection, immune-medicated diseases, and idiopathic causes [67].

A 1992 case report describes three related GSD with neurogenic muscular atrophy, demyelination, and Wallerian degeneration of the peripheral nerve fibers [86]. This is the first report to suggest a possible hereditary condition in GSD. The authors compared this peripheral nerve finding in the GSD with hereditary motor and sensory neuropathy (HMSN) type II, which is associated with age and pathological changes in man.

Braund described some neuropathies and polyneuropathies, including a German Shepherd Dog myelopathy [87, 88]. The neuropathies are usually bilateral with a distal limb muscle distribution as found in giant axonal neuropathy in the GSD and distal symmetrical polyneuropathies in large-breed dogs. Most dogs exhibit a chronic clinical course. Some of the clinical signs of classical motor neuropathy are reduced or absent reflexes, muscle tone, and neurogenic muscle atrophy. Other neuropathies result from exposure to environmental toxic agents (ie. vincristine, thallium, lead, organophosphate, aminogycosides). Many of the responses are acute in nature. For those that are chronic, the lesions include distal degeneration of motor nerves, beginning in the periphery and then following the motor nerves into the spinal cord. Braund notes that dogs tend to be more resistant than cats to the neurotoxic effects of these exposures.

Neurologic Toxicants in Environment

Veterinary Insecticides: Dips, Sprays, Pour-ons, Shampoos, and Collars

During the late 1980s and early 1990s, many insecticides and repellants were used on the MWD in the forms of dips, sprays, pour-ons, shampoos, and collars and one oral insecticide. The products used were from the insecticide classes of pyrethroids, organophosphates, carbamates, growth regulators, and an amidine product for mites. By 1997 most of the dipping performed at the DoD MWD Veterinary Services had been limited to treating specific mite problems and newer pour-on and spray products were used for flea and tick control and as fly/mosquito repellants.

The formulations of the pyrethroids were pyrethrins, permethrin, and allethrin as dusts, dips, sprays, and shampoos [89]; among the organophosphates, cythioate (orally), fenthion (pour-on), chlorpyrifos (collar, dip, and spray), and Paramite® (dip). Shampoos, dusts, and collars containing carbaryl or a carbamate generation ingredient were the carbamate formulations. Of all the organophosphates, the clinical toxicity response to fenthion is reportedly different. Clinical signs of toxicity develop over an extended period of time. Onset of chronic weakness, the primary demonstrating sign, is often delayed [85]. Amitraz is a dip, without significant AChE inhibitory activity, that is specifically used for demodex and scabies mite infections. Methoprene, is an insect growth regulator, used in conjunction with other insecticide products that work at the larval and maturing stages of insects; methoprene has not been shown to have detrimental affects in humans or dogs [89-91]. One of the newer products, is the pour-on Frontline®, fipronil, a pyrazole insecticide, which is listed as a low toxic substance that has no known neurologic effects as a topically applied insecticide in the dog [92]. A chronic progressive peripheral neuropathy associated with the exposure to pesticides has been reported in cats, but not in dogs [85].

Known Agents Used During Gulf War

Environmental toxicants that were relevant during the GW would include a list of volatile organic compounds (i.e. fuels, solvents, paints), heavy metals, and pesticides. Since many volatile organic compounds only have an internal half-life of 24 to 48 hours in the human, only acute neuropathy symptoms are generally seen. However, an association of chronic exposure to n-hexane and methyl n-butyl ketone, both components of solvents and glues, have been documented with progressive peripheral neuropathy[93-97].

The heavy metal exposures of concern during the GW include chromium, cadmium, and lead. These are common components of paints and armor plating; soldiers would be exposed to them occupationally in the regular work environment, during preparation for war, in the theater of war, and during combat. Inhalation or ingestion of these metals have been associated with peripheral neuropathies in humans [98-101].

Sixty-four pesticide products were dispensed in the logistics channels during the GW. These products were in the classes of: organophosphates, methyl carbamates, organochlorines, pyrethroids, and *n,n*-diethyl-m-toluamide [3, 10, 14]. Of these products, 15 were pesticides of potential concern that were included in a thorough health risk assessment by the DoD Environmental Exposure Report Board [14]. These products included azamethiphos (1% crystal), bendiocarb (76% solid), chlorpyrifos (19% and 45% liquid concentrates), diazinon (48% liquid), dichlorvos (20% plastic strip), DEET (33% cream & 75% liquid), lindane (1% powder), malathion (57% and 91% liquid), methomyl (1% crystal), permethrin (0.5% aerosol), d-phenothrin (2% aerosol), and propoxur (14.7% liquid). The investigative health risk assessment on pesticide exposure concluded that the possibility exists that some troops were exposed to pesticides that exceeded reasonable risk-based levels

of concern; however, the majority of veterans probably were not exposed above these levels of concern [14].

Additional exposures occurred after Iraqi forces left Kuwait, leaving behind burning oil wells. The U.S. Army Environmental Hygiene Agency performed a health risk assessment to evaluate the levels of risk posed by pollution produced by the burning wells [102]. Exposure agents included particulates, gases, and combustion products (i.e. metals, volatile organic compounds, and polycyclic aromatic hydrocarbons). The report concluded that the predicted non-cancer risk did not differ between any of the monitoring sites in Kuwait and Saudi Arabia. Inhalation of volatile organics (primarily benzene) contributed to over 99.0 percent of the non-cancer risk.

Other Neurologic Agents

Other environmental agents that could be present in a war environment were discussed in an article reviewing environmental exposures to working dogs found in the aftermath of the New York World Trade Center bombing [103]. These toxicants could be found as solids, liquids, particulates, and gases and may be ingested, inhaled, or absorbed by physical contact. The agents that may result in neuropathic degeneration include n-hexane, lead, organic mercury, and thallium. Other agents, may produce neurologic symptoms, but they primarily produce acute physiologic changes to other primary organs, severe illness, and death.

Preliminary Studies

A 1996 cross-sectional study took a sample of 512 dogs from part of the GW-era MWD cohort and looked at six morbidity and nine mortality outcomes. The study

demonstrated that for the outcome of Service related illnesses or pathologic conditions at death, there were no differences or there were protective associations among those MWD that deployed to SWA compared to those that did not deploy [104]. These results were attributed to the 'healthy working dog effect.' However, peripheral neurologic disease mortality (OR = 8.3, 95% CI 3.0, 21.5) was found to be statistically different. The prevalence levels of neurologic disease were reported at 7.4 percent overall, ranging from 5.0 percent among the non-exposed group to 31.0 percent among the exposed MWD. Limitations of this study included the method used to define the mortality outcome categories and the possibility of misclassifying individuals.

The DoD Veterinary Service Activity initiated a prospective GW MWD cohort study in December 1996 (APPENDIX B), to further investigate the potential relationship between deployment during Operation Desert Shield/Storm and the morbidity and mortality outcomes. A military veterinarian boarded by the American College of Veterinary Internal Medicine redefined the mortality groups into 20 categories by using specific determinants for classification of each mortality outcome [61]. In the 2001 study, causes of death and euthanasia of MWD that had died between 1993 and 1996 were retrospectively examined. Twenty specific categories were identified and used to assign a single primary cause of death for each MWD. Of 927 MWD records evaluated, approximately 15.6 percent who died or were euthanized had a neurologic related disease. Among the BSD and GSD, respectively, 13.7 percent and 19.4 percent had died of neurologic related disease [61].

CHAPTER 2: MATERIALS AND METHODS

Research Design

This study used an observational cohort design, part of which was retrospective in nature and a part that was prospective. The study provided data to determine the association of Southwest Asia (SWA) deployment and neurologic disease outcomes in the Gulf War (GW) military working dog (MWD) cohort. A prospective GW MWD cohort study was designed and initiated in December 1996 (Appendix B) to investigate the potential relationship between deployment during Operation Desert Shield/Storm and morbidity and mortality outcomes. The prospective cohort study added a focus on peripheral nerve pathology and required other neurological measures during the final disposition physicals and necropsies.

Research Question and Study Aims

As a result of studying the literature and preliminary studies that have focused on the GW, the primary research question was: Is Gulf War deployment to Southwest Asia a risk factor for peripheral nerve disease (PND) among military working dogs?

Using this research question, the following specific aims were developed:

Specific Aim 1. Using the total GW MWD cohort:

a. Determine the neurologic mortality incidence.

b. Determine the association of neurologic mortality with SWA deployment, controlling for the possible confounding factors of age, breed, gender, and branch of Service.

Specific Aim 2. Using the Prospective MWD cohort:

a. Determine the PND incidence.

b. Determine the association of PND with the SWA deployment, controlling for the possible confounding factors of age, breed, gender, hypothyroid status, occupation, and branch of Service.

Specific Aim 3. Determine the neurologic mortality differences within the SWA deployed MWD cohort with respect to the level of exposure whether associated with deployment location, arrival and departure periods, or length of deployment?

It was hypothesized that among the GW MWD cohort there was no difference in neurologic mortality or PND between those MWD that deployed to SWA and those MWD that did not deploy to the SWA theater of war.

Study Population

GW MWD Cohort

Entry into GW MWD cohort was defined as a MWD that was: in the MWD inventory between the dates of 1 August 1990 and 31 December 1991; assigned to a military unit between 1 August 1990 and 1 December 1991; and died after 4 September 1990.

Approximately 2,398 MWD were in the inventory as of April 1991 [105]. About 275 dogs were in training or used in training at Lackland AFB and therefore were not included in the cohort. The study cohort included 2,123 dogs (Figure 2-1). By the end of the observation period on 30 June 2001, 43 dogs survived and 4 dogs had been lost to follow-up.

All dogs were assumed to have been in good health and receiving the same preventive medicine and health care treatments. These treatments included vaccinations, heartworm and

intestinal worm preventatives, and insect pesticide/repellant treatments. None of the dogs received botulism or anthrax immunizations.

Prospective MWD Cohort

The Prospective MWD cohort included all dogs of the GW MWD cohort that met the entry criteria and remained alive on 1 January 1997. In January 1997, 39 MWD that met the exposure criteria and 612 MWD that met the unexposed criteria were alive. These MWD were followed concurrently and evaluated through 30 June 2001. Of the 651 MWD in the prospective cohort, 347 had peripheral nerve samples sent to an independent pathologist for examination. Of the remaining 304 dogs, 43 dogs were alive at the end of the observation period, 75 dogs did not have necropsies performed, 6 dogs died prior to the activation of the pathology contract, and 180 dogs had necropsies, but the additional nerve sampling and evaluation had not been performed.

Exposure

The exposure location was defined by the place of assignment between 1 August 1990 and 31 December 1991. If the MWD was moved or reassigned in that time period to a different location, then the location at which the MWD was stationed on 1 May 1991 was used as the location of assignment. If the dog became deployable between May and December 1991 from a training location then the dog was coded for this first assignment after training. In order for a dog to be considered exposed, the MWD must have deployed and physically resided in the countries of Saudi Arabia, Kuwait, Bahrain, Qatar, or United Arab Emirates for at least 30 days between 1 August 1990 and 31 December 1991. A deployment to the region before or after this time period was not considered an exposure for

this study. There were 126 dogs identified as deploying to SWA, and 1,997 MWD that did not deploy.

Sample Size and Power Estimates

Published PND incidence and prevalence levels are practically nonexistent for the MWD. Reported neurologic disease prevalence levels were between 6.0 and 16.0 percent among the general MWD population, but Vander Wyst reported prevalence levels of 5.0 percent among the non-deployed group and 31.0 percent in those that deployed from her sample of the GW group [56, 61, 104]. Sample size and power calculations were performed using PS© Power and Sample Size Calculation software [106] for the full and prospective GW MWD cohorts.

To determine sample size calculations for survival analysis, power was set at 80 percent, estimated median survival time was 100 dog-months, α=0.05, and follow-up time was assumed to be 131 months (17 months recruitment and 114 months observation). The calculated sample sizes and hazard ratios (HR) may be compared to the available population within each cohort (Table 2-1). Within the GW cohort and the prospective cohort sufficient numbers exist that well exceed the power of 80 percent with the given numbers in the populations.

The GW cohort had sufficient numbers to detect an elevated HR between unexposed and exposed as low as 1.4, and reduced HR as high as 0.7. For the prospective cohort, the available dogs allowed for detecting elevated HR as low as 2.0 and reduced HR as high as 0.6 between unexposed and exposed dogs. Accounting for the lack of data and using the

existing dogs, it was sufficient to detect a HR as low as 2.2 and as high as 0.5. These HR are very similar to the former eligible population calculation.

Data Sources

Data Quality and Management

Certain demographic and identifying variables that had been assigned and used throughout each animal's life were used to organize and maintain quality control of the data. These include: a **tattoo number,** an alphanumeric permanent mark placed in the earflap and/or inside of the thigh of the dog when the animal was added to the MWD inventory; and the **name** given to the dog at the time of procurement.

All MWD receive semi-annual physical examinations in accordance with military standard operating procedures [107]. Beginning in 1997, when the examining veterinarian determined that the animal was no longer physically fit for duty, the MWD was transported to the DoD MWD Veterinary Services (DODMWDVS), Lackland AFB for a final disposition physical. The final disposition physical examination included the following tests: complete blood count, serum chemistry panel, serum acetyl cholinesterase levels, urinalysis, fecal exam, thyroid panel, electrocardiogram, electromyogram, electroencephalogram, a nerve conduction study, radiography, and a clinical neurologic examination. If euthanasia* was elected to relieve pain and suffering or if the MWD died, a full necropsy was performed. Moore's mortality categorical classification was used to designate the final clinical mortality outcome for each MWD [61]. Tissue samples from all organ systems were sent to Armed

* Other disposition outcomes include MWD being kept as training aides, sent back to duty, transferred to other government agencies (i.e. Immigration and Naturalization Service, Federal Bureau of Investigation, Customs, or Police Depts.), or as of November 2000, adopted as a pet.

Forces Institute of Pathology (AFIP) for pathologic examination and archiving [108]. For all prospective GW MWD cohort eligible dogs, additional samples of the *biceps femoris* and *triceps brachii* and the tibial and radial nerves were collected and sent to a non-DoD affiliated pathologist at Veterinary Neurological Consulting Services, Dadeville, Alabama for pathologic evaluation. All samples were masked for exposure status.

Study data was abstracted from the dog's medical, clinical, and pathologic records and death certificates maintained at the DODMWDVS and the AFIP, Washington, DC. These records were in one of three formats: hard paper copy, microfiche, or electronic document format. Additional information was extracted from archived GW deployment files compiled by veterinary services officers. Data abstraction sheets (Appendix C) were used to extract the information directly from the records. All GW MWD medical records contain a MWD Medical Record Microfilm Data Sheet (Appendix D) and a MWD Gulf Study Checklist (Appendix E) that assisted in the maintenance and completion of all necessary examinations and testing. All the data were collected and abstracted from the records by the principal investigator and two other trained epidemiologists. A trained computer database operator entered data into a FoxPro® environment computer database specially designed for the long-term data management of MWD medical and service records at DODMWDVS.

The nerve conduction data from the final disposition physical exam were digitally archived by the veterinarian performing the procedure using a Nicolet Viking IV P™ monitoring and diagnostic machine. The data was imported into the FoxPro® database. All information was reevaluated and assessed for input error and cross-matched with the data abstraction sheets.

The variables are described below. A variable code sheet is included in Appendix F.

Main Predictor Variables (Exposure)

Deployment Status: Deployment status was the primary exposure variable of this study. Different attributes to deployment were evaluated for their effect on the outcome measures.

Southwest Asia Deployed: This variable was defined as a MWD that must have deployed and physically resided in Saudi Arabia, Kuwait, Bahrain, Qatar, or United Arab Emirates for at least 30 days between 1 August 1990 and 31 December 1991. This variable was coded as a binomial, yes or no, and used as an exposed/unexposed variable for the GW and prospective analysis.

Assignment Location: For the GW and prospective cohorts, this variable had three categories based on being stationed in SWA, outside the continental U.S. (OCONUS) other than SWA, or in the continental U.S. (CONUS).

The assignment location for the exposed cohort was collected as the exact base camp location of the MWD as could be identified in the medical and GW records. There were ten identified SWA locations for 126 dogs. This variable was then collapsed to look at two groups: those stationed in the Kingdom of Saudi Arabia and those dogs stationed in other SWA countries compared to the unexposed group.

Length of SWA Deployment: This variable was listed as the number of days the dog resided in SWA between 1 August 1990 and 31 December 1991. A quartile variable was generated to evaluate length of deployment as a dose exposure. The non-deployed population was the comparison group.

Arrival and Departure Periods: These variables defined the period in which the dog arrived to and departed from SWA. There were three periods: the pre-war period (1 August 1990 - 31 December 1990), the war period (1 January 1991 - 31 March 1991), and the post-war period (1 April 1991 - 31 December 1991); the comparison group was the non-deployed population.

Outcome Variables

Neurologic Mortality: This variable identified a neurologic final cause of death as defined by Moore (Appendix F) [61]. For the GW and exposed cohort, this variable was used as the measure of neurologic disease. For the purpose of this study, death/euthanasia attributed to peripheral nerve related illnesses was categorized as "NEURO." This category was chosen if the MWD was euthanized for a chronic history of presenting symptoms and clinical signs that included a crouched stance, a short-stride walk, or a 'bunny hop' gait, with weakness or flaccid paresis, and diminished to absent nerve reflexes [70]. Those dogs that showed any evidence of degenerative joint disease in any of the appendages or along the spinal column were carefully evaluated for nerve involvement. However, clinical assessment of the recorded primary presenting symptoms and signs used to choose euthanasia were deemed important in differentiating between orthopedic and neurologic causes of death.

Peripheral Nerve Histopathology: Nerve histopathology is the gold standard for identifying PND [38, 64]. The Veterinary Neurological Consulting Services based PND on microscopic examination of teased nerve fibers and calculation of the incidence of teased histopathologic changes. Normal was based on up to 20 percent changes if >12 years of age or up to 15 percent changes if ≤12 years of age. Neuropathy was based on >20 percent if >12

years of age or >15 percent changes if ≤12 years of age [68, 69, 109]. This measure was a dichotomous variable identified as affected or not affected.

Potential Confounding Variables

Demographic Variables:

Gender: Gender in the MWD has been shown to influence different morbidity and mortality outcomes [52, 53], yet the effect on neurologic outcomes is less understood. It was defined as female-spayed, male-neutered, and male-intact. Female-spayed was the base comparison group.

Breed: Breed has been associated with some neurologic diseases, particularly those that are heritable [52, 86]. The majority of the MWD cohort consisted of Belgian Shepherd Dogs (BSD) and German Shepherd Dogs (GSD) by breed. Breed was coded as BSD, GSD, and other. BSD was used as the primary comparison group.

Age: Age was expected to influence peripheral neuropathy. Many pathological changes occur within the body associated with age and can effect neurologic symptoms, signs, and pathologic nerve changes [52-54, 68, 69]. Two age variables were calculated: first, **age at death** was determined from the date of birth and the date of death. Second, **age at entry** to the cohort was calculated from the date of birth and the date when the MWD became deployable between 1 August 1990 and 1 December 1991. The multivariate model was adjusted for age by using age at entry.

Survival Time: The amount of time contributed by each dog during observation was calculated from the time that the dog entered the cohort to the date which it was either lost to

follow-up, the date of death, or 30 June 2001 if the dog survived to the end of the observation period. The units were calculated in months.

Service Assignment: After training, each MWD was assigned to a particular military Service branch. This is a categorical variable, which includes U.S. Air Force, U.S. Army, U.S. Navy, and U.S. Marine Corps. The Air Force was used as the comparison group.

Occupation: A MWD receives specific training and certification, including: patrol (included scout dogs), explosive detection, and/or drug detection. Most detector dogs were dual trained as a patrol dog. The occupation variable was divided into three categories, patrol, patrol/drug, and patrol/explosive. The patrol category was used as the comparison group.

Thyroid Panel Results: Decreased thyroid hormone levels have been reported to occur concurrently with degenerative nerve changes or hypothyroid related neuropathies [87]. These continuous values included total thyroxine (T4), free T4, and canine thyroid stimulating hormone (cTSH; thyrotropin) values. Reported normal values by the IDEXX Laboratory on the report forms were: total T4: 1.0 to 4.0 μg/dl; free T4: 0.7 to 2.5 ng/dl; and cTSH: 0.05 to 0.42 ng/ml. Some free T4 reports were recorded in nmol/L and were converted to ng/dl by dividing by the conversion factor of 12.87 [78].

Hypothyroid Status: Hypothyroid status of the dogs was determined using the total T4, free T4, and cTSH levels. Recognized hypothyroidism was defined as below normal levels of total T4 and free T4 with above normal cTSH levels [72, 80, 81]. The MWD that fit these parameters were categorized as hypothyroid; all others were considered normal.

Other Variables Collected

AFIP Results: By standard operating procedure, all MWD that died during military service were necropsied and samples of all organ systems were sent to AFIP, Washington, DC [108]. The major clinical histopathologic findings were cataloged using the Systemized Nomenclature of Medicine (SNOMED® International) for Human and Veterinary Medicine system, endorsed by the College of American Pathologists, and is similar to the International Statistical Classification of Diseases and Related Health Problems format. The SNOMED numerical categories that included the peripheral nerve system disorders were DA-40000 and DA-43000 through DA-45054, inclusive [110].

Nerve Conduction Velocity: The conduction velocity (meters/second; m/s) of the tibial and ulnar nerves.

Cholinesterase (AChE): Cholinesterase is used to diagnose exposure to neurotoxic pesticide agents. Normal levels are reported at 1.2 to 3.0 IU/ml [83].

Data Analysis

The outcomes of interest were neurologic mortality and PND incidence among the full and prospective GW MWD cohorts, respectively. Data analysis involved the description of each independent variable in relation to each main exposure measure and in association with neurologic mortality and PND.

Neurologic mortality and PND incidences were calculated using unadjusted two-way comparisons with dog-time; unadjusted hazard ratios were computed with Cox regression. Each categorical variable was expressed as unadjusted rates and rate ratios with 95 percent confidence intervals (CI). Significance values were determined using Fisher's exact

methods. The primary exposure variable comparisons were made against being assigned to CONUS (for GW and Prospective cohorts) or the non-deployed (for SWA deployed group). The rate of dying from or being a case of PND for independent variables was compared to that of being a Belgian Shepherd Dog, a spayed female, assigned to the Air Force, a patrol dog, or normal thyroid function.

Comparisons of time variables (age at entry, age at death, and exposure time) between groups were performed with the Student's t-test. Comparisons of two or more proportions of nominal variables were analyzed with the Pearson's chi-square.

Multivariate analysis was performed using right-censored data and Cox regression to control for and measure the effect of each exposure and independent variable on the outcome. Dummy variables were created for each categorical variable. The failure event was a dog that died with evidence of neurologic disease: the neurologic mortality variable (used for GW cohort) and the peripheral nerve histopathology variable (used for Prospective cohort) served as the event indicator. Observations were censored at time of death due to a non-neurologic cause, loss to follow-up, or survival to the end of the study period.

Estimated Schoenfeld and scaled Schoenfeld residuals and testing the linear slope of the plotted residuals over time were used to prove the proportional hazard assumption for each variable. This test produced a $\chi 2$ statistic for each variable based on the observed and expected survival probabilities. Additional examination of the plotted estimated -ln (-ln) survival probabilities was used to verify the proportional hazard assumption. Non-proportional variables were included in the model by stratification using the stratified Cox procedure. Logistic regression was used to evaluate the exposure of length of deployment to

SWA to alleviate any conflict that this time variable may have with follow-up time in the Cox model.

Interaction terms were evaluated between each independent variable and the primary exposure variable. Significant change shown by a significant Wald statistic, and verified with the likelihood ratio difference between reduced and full models, was used to evaluate the importance of each variable and interaction term in the model. All independent variables were included in the final models to control for and examine their effect in relation to the neurologic outcome. For all analyses, values $P<0.05$ were considered significant. STATA® statistical software was used to perform all statistical analysis [111].

Ethical Considerations

The project received approval from the Office of Research, Uniformed Services University of the Health Sciences under the Project Number T087RK-01. An exemption from evaluation by the Institutional Review Board and the Institutional Animal Care and Use Committee (IACUC) was granted for this study. This study protocol did not use any additional sampling of animal tissue, fluids, or other bodily items. The MWD medical, clinical, pathological, neurological, and deployment records were used only to abstract and consolidate existing objective data and make interpretive diagnostic outcomes. Furthermore, this project did not use additional animal or human subjects or sampling.

This project was subordinated to and supported by the ongoing AFIP Protocol UBAD2, "Indicators of Human Disease from Persian Gulf War Service: A Study of Military Working Dogs deployed in Operations Desert Shield/Storm" that was initiated in early 1997. The AFIP Research committee and IACUC conditionally approved it on 18 February 1997

and 16 June 1997, respectively, with final approval made 5 September 1997 (Appendix B). The procedures were reviewed and found not to extend past the normal and ethical use of the MWD approved under military regulation and standard operating procedures which define the laboratory, disposition examination, and necropsy sampling techniques that were used and followed [107, 108].

Study Investigators

LTC Kelly G. Vest, USA, VC was responsible for the overall design, management, data abstraction, and data analysis for this study. MAJ Linda D. Harris, USA, VC performed the initial testing of the abstraction sheets. Assistance in locating records and data abstraction was received from MAJ Linda D. Harris, USA, VC and LTC Kay D. Burkman, USA, VC. Mrs. Isabel Castro-Perez entered data into the computer.

CHAPTER 3: RESULTS

Gulf War Military Working Dog Cohort

Descriptive Analysis

The Military Working Dog (MWD) Gulf War cohort was composed of 2,123 dogs, 57.9 percent Belgian Shepherd Dogs (BSD), 34.1 percent German Shepherd Dogs (GSD), and 8.0 percent of 14 other breeds (Table 3-1). The cohort consists of 58.7 percent gonad intact males, 26.5 percent neutered males, and 14.8 percent spayed females. The U.S. Air Force (USAF) was the primary user of MWD (48.0%) followed by the U.S. Army (USA; 29.1%), U.S. Navy (USN; 17.4%), and U.S. Marine Corps (USMC; 5.6%). Deployment status at the time of the Gulf War (GW; exposure) for these dogs was based on three assignment locations: the continental United States (CONUS: 45.1%), outside the continental U.S. (OCONUS: 49.0%), and Southwest Asia (SWA: 5.9%).

The distribution of the breeds was similar across locations except a slightly higher proportion of other breed dogs were assigned to OCONUS locations compared to CONUS (Table 3-1). Gender distribution was similar across locations between males and females, although when males were split into gonad status strata, a significant difference ($p=0.015$) existed in the distribution of neutered and intact males among OCONUS and SWA dogs compared to CONUS stationed dogs. By branch of Service assignment, the distribution of the dogs deployed to SWA was significantly different compared to CONUS and OCONUS MWD ($p<0.001$). SWA deployed MWD were primarily USAF dogs (86.5%).

The average age of the MWD upon entry into the cohort was 69.1 months, living to an average age of 125.0 months at death (Table 3-2). Both GSD and other breeds entered the

cohort at an older mean age; other breeds died on average 4.8 months older than BSD. Neutered males were younger than spayed females at entry and intact males were older. Both spayed females and neutered males died at an older average age than intact males.

The mean age at entry was similar among the Services except for the USN dogs that were younger at entry and died younger. On the other hand, USMC MWD lived longer on average than dogs of other Services. The average entry age of the OCONUS and SWA dogs was older than CONUS dogs. Compared to CONUS dogs, OCONUS dogs lived on average four months less; SWA dogs lived six months longer.

Neurologic mortality accounted for 10.6 percent of all cohort deaths, behind appendicular degenerative joint disease, old age, and neoplasia. The distribution of cases and non-cases of neurologic mortality was similar across the demographic factors of breed, Service, and assignment location (Table 3-3). Neutered males made up a higher proportion of cases than of non-cases ($p=0.015$). There was no difference in the mean age at entry between cases and non-cases. Cases lived to an older average age than non-cases.

Neurologic Mortality Rate

A total of 118,686 dog-months were contributed in the cohort with 226 neurologic deaths, leading to an incidence density of 1.9 cases per 1,000 dog-months (Table 3-4). The highest incidence was shown in GSD with 3.3 cases per 1,000 dog-months, which was 2.2 times (rate ratio=2.16, RR; 95% C.I. 1.62, 2.85) the rate among BSD. Males were not different from the females, nor did the USA, USN, or USMC dogs differ statistically from the USAF dogs, even though the USMC incidence was 21.0 percent higher (RR=1.21; 95% CI 0.67, 2.06). The incidence rate was similar for the dogs assigned to CONUS and

OCONUS. Dogs deployed to SWA had a 33.0 percent increase in neurologic mortality, relative to dogs deployed to CONUS, but this difference was not significant (RR=1.33; 95% CI 0.77, 2.19).

Multivariate Analysis

Proportional Hazards Assumption:

Using the proportional hazards assumption test to screen the variables, all the variables except breed met the proportional hazards assumption criteria with non-significant χ^2 levels. Adjusting for gender, age at entry, Service, and assignment location, breed violated the proportional hazards assumption with a χ^2=9.34, 1d.f. (p=0.002).

Graphically, the unadjusted log-log survival probability plot (Figure 3-1) shows a quickly reducing survival probability for the GSD relative to the base comparison breed, BSD. The survival curves are not parallel and converge with time. This becomes more evident in the adjusted model (Figure 3-2), which shows the survival curves crossing at approximately 100.0 months of analysis time, indicating non-proportionality. The stratified Cox regression method was used to correctly adjust for breed in the model.

Neurologic Mortality Rates for Demographic Variables

The adjusted rate ratios (RR) for each demographic variable can be seen in Table 3-5. In comparison to the BSD, GSD experienced 2.1 times the rate (RR=2.11; 95% CI 1.55, 2.88) of dying from neurologic disease when adjusted for gender, assignment location, Service, and age. The adjusted rate for intact males was 35.0 percent higher than the rate for spayed females (RR=1.35; 95% CI 0.90, 2.04), even after stratifying by breed and adjusted for assignment location, Service, and age at entry. The adjusted rates for branch of Service

were not significant. Age has the most significant effect on neurologic mortality (RR=1.05; 95% CI 1.04, 1.06). For each month increase in age, the neurologic mortality rate increases by 5.0 percent.

Southwest Asia Neurologic Mortality Rates

The adjusted rate of neurologic mortality for SWA was practically equal to the unexposed (CONUS plus OCONUS) dogs (RR=1.00; 95% CI 0.61, 1.63) when stratified by breed and adjusted for gender, Service, and age (Table 3-6). Splitting the non-deployed dogs into CONUS and OCONUS strata, the adjusted rates for SWA and OCONUS dogs were similar to CONUS dogs (RR=1.04, 1.08, and 1.0, respectively). Further expanding OCONUS into major military geographic regions, the adjusted rates across all the exposure groups compared to CONUS were not significantly associated with neurologic mortality.

1997 Prospective Gulf War Military Working Dog Cohort

Descriptive Analysis:

The 1997 prospective GW MWD cohort includes 651 eligible dogs (Table 3-7). These dogs were primarily composed of BSD (79.0%), followed by GSD (14.4%) and other breed dogs (6.6%). The majority of the dogs were male (84.5%) followed by spayed females (15.5%). The males were approximately equally split in distribution between neutered and gonad intact individuals (44.2% and 40.3%, respectively). Most of the dogs were assigned to USAF (45.0%) with the USA (26.4%) and USN (23.8%) approximately equal, followed by the USMC dogs (4.8%). Information on MWD occupation and hypothyroid status was available for the prospective cohort. Of the 651 dogs, one dog's occupation remained unknown, 17 were drug detectors only, one dog was an explosive detector only, and all

others (n=632) were patrol (42.4%) or dual trained as patrol and drug or patrol and explosive detectors (38.6% and 19.0%, respectively).

The distribution of the breed and gender across the assignment locations of OCONUS or SWA compared to CONUS showed no significant differences. Service assignment, on the other hand, was not distributed similarly ($p<0.001$). As in the full cohort, USAF dogs predominated in the SWA deployed group.

The distribution among occupation was also significantly different between location strata ($p<0.001$). OCONUS stationed dogs had a higher proportion of patrol dogs and a lower proportion of patrol/drug dogs than CONUS assigned dogs. The occupation distribution of SWA dogs was approximately equally distributed.

The prospective cohort's mean age at entry was 41.5 months; they died at a mean age of 139.8 months (Table 3-8). There are no differences in the mean age at entry among the different demographic variables. Spayed females, USMC dogs, and those deployed to SWA were older at entry and lived to an older age at death.

The proportion of deaths that were attributed to neurologic mortality in the prospective cohort was 12.0 percent (Table 3-9). The neurologic mortality rate was 1.2 neurologic mortality cases per 1,000 dog-months, which was less than reported for the full GW cohort. Breed was not proportional for the hazard model. The adjusted rates for neurologic mortality showed no significant differences among breed, gender, Service, or assignment location. Age reflected a significant effect on neurologic mortality with an adjusted RR = 1.06 (95% CI 1.04, 1.08).

Peripheral Nerve Disease

Peripheral nerve disease (PND) was determined from nerve samples taken at necropsy of the MWD and examined by a veterinary pathologist. Of the 651 eligible prospective cohort individuals, 347 (53.3%) dogs had nerve samples and 304 (46.7%) did not have nerve samples submitted (Table 3-10). The demographic characteristics between these two groups were not proportionally different in breed, gender, and occupation. The MWD that did not have a nerve sample submitted had proportionally more USA and fewer USN dogs than the group that was sampled ($p = 0.018$). The location distribution was different, with the group without nerve samples composed of proportionally more dogs from OCONUS and the group with samples weighted with those dogs deployed to SWA (<0.001). The MWD with nerve samples were older at entry ($p<0.001$) but lived to a similar age at death than the unsampled group.

The distribution of PND among the demographic factors was similar between cases and non-cases (Table 3-11). Slight, but not significant, variation in distribution exists within gender and service strata comparisons of cases and non-cases. Finally, PND cases were similar in age at entry, but were older at death ($p<0.001$).

Further examination of the 347 dogs with biopsies showed 17.0 percent of them were dogs that died from a neurologic condition, 39.0 percent died with degenerative orthopedic disease, and the remaining (44.0%) died of other processes. Of the 121 PND positive dogs, 21.0 percent were neurologic mortality cases, 37.0 percent were dogs with degenerative orthopedic disease, and the remaining (42.0%) MWD died of other causes.

Peripheral Nerve Disease Incidence

Among the prospective cohort, 32,810 dog-months were contributed through the study period for the 347 dogs with nerve samples (Table 3-12). The overall crude incidence for the cohort was computed as 3.7 cases/1,000 dog-months. The highest PND incidence was found among spayed females and USMC dogs (4.8 and 6.2 cases/1,000 dog-months, respectively).

Multivariate Analysis

Proportional Hazards Assumption

The proportional hazards assumption was not satisfied for breed similar to the full GW cohort. Using the Cox proportional hazard assumption test, the $\chi 2 = 2.53$, $p= 0.1119$ for GSD was borderline significant. However, the adjusted log-log survival probability plot (Figure 3-3), clearly was not proportional as the GSD survival probability line crossed the BSD survival line at approximately 100 months of analysis time. The stratified Cox model was used to correctly adjust for breed.

Rates of the Demographic Variables

Using the stratified Cox model, stratifying by breed and adjusting for assignment location and the other remaining factors (age, gender, Service, occupation, and hypothyroid status), each of the variables was evaluated for the effect on PND (Table 3-13). Gender, branch of Service, occupation and hypothyroid status were not significantly associated with PND. On the other hand, age was an important covariate in the model, influencing a significant 7.0 percent increase rate of PND (RR=1.07; 95% CI 1.05, 1.08) for each one month increase in age.

Southwest Asia Peripheral Nerve Disease Rates

The adjusted rate ratios for SWA deployment show no significant association with PND (Table 3-14). SWA deployment becomes similar to the CONUS dogs (RR= 1.02; 95% CI 0.49, 2.13) when stratified by breed and adjusted for gender, Service, occupation, hypothyroid status, and age.

Military Working Dogs Deployed to the Gulf War

Descriptive Results

The exposed MWD were the 126 dogs that deployed to SWA for at least 30 days between August 1990 and December 1991. By breed, this group was composed of 61.1 percent BSD, 31.0 percent GSD, and 7.9 percent other breeds; 83.3 percent were males and 16.7 percent spayed females (Table 3-15). The Service distribution was USAF (86.5%), USMC (7.9%), USA (4.8%), and USN (0.8%). By MWD occupation status the dogs were patrol/explosive detectors (40.3%), patrol/drug detectors (31.5%), and patrol-only (28.2%) dogs. The average age at entry was 70.5 months, and their mean age at death was 132.4 months (Table 3-2).

Most of the dogs that deployed to SWA were stationed in Saudi Arabia (86.5%). The remaining (13.5%) were stationed in other countries in the region including Bahrain, Kuwait, Qatar, and United Arab Emirates. Their home stations were either CONUS-based (91.3%) or European stations (8.7%). Most dogs deployed before and during the war (93.7%) and departed during and after the war (97.5%).

The average length of stay in SWA was 133.2 days, ranging from 30 to 304 days. German Shepherd Dogs on average stayed longer in theater, as did spayed females, USAF

dogs, and patrol/drug dogs. The USMC dogs stayed an average of 101.3 days, which was less than the other Services.

The dogs assigned to areas in Saudi Arabia spent a longer time in theater than those in other SWA countries. When considering the period in which the MWD arrived and then departed the region, arrival period was inversely correlated ($r = -0.49$, $p<0.001$) with time spent in theater. The MWD entering before war stayed the longest. Those arriving during and after the war spent less time in SWA. Departure was not correlated with length of stay.

Age comparisons between cases and non-cases of neurologic mortality showed that cases were slightly older at entry and death (Table 3-16). Cases stayed in SWA for a longer mean number of days than non-cases. None of these differences were statistically significant.

Neurologic Mortality Rates

During 7,803 dog-months of observation, 19 (15.1%) of the 126 SWA-exposed dogs died or were euthanized due to neurologic disease; the incidence density for neurologic mortality among this cohort was 2.4 cases per 1,000 dog-months (Table 3-17). All cases were USAF dogs. The rate was highest among other dog breeds, spayed females, and patrol/explosive detector dogs. No statistical differences were found among the covariates.

To evaluate neurologic mortality incidence by time-in-theater, the CONUS and OCONUS unexposed group of the GW cohort was used in comparison (Table 3-18). Incidence of neurologic mortality was found to be highest for dogs not assigned to Saudi Arabia, pre-war arrival, post-war departure, staying in SWA for three periods, and residing in SWA for >176 days.

There was a decreasing rate of neurologic mortality from one arrival period to the next and an increasing rate for departure over the same periods. There was an increasing rate with increasing number of periods spent in SWA. If the length of time spent in SWA was divided into quartiles, there was an increasing rate of neurologic mortality with the longer a dog spent in theater.

Multivariate Analysis

The stratified Cox model, stratified by breed and adjusted for gender, Service, and age, was used to evaluate deployment location, periods of arrival and departure, and number of periods residing in SWA (Table 3-19). Dogs assigned to SWA countries other than Saudi Arabia, and dogs that stayed in SWA for three periods had the highest adjusted rates. None of the adjusted comparisons were significantly associated with neurologic mortality (Figures 3-4 to 3-6).

Using logistic regression, the amount of time that the dogs spent in SWA showed an increasing trend related to neurologic mortality (Table 3-20). Using quartiles for the time spent in SWA showed increasing odds of neurologic mortality with increased time spent in SWA. Although there is not a significant effect at any one quartile, there is a tendency for an increasing trend of neurologic mortality to move toward significance at the fourth quartile ($p=0.047$). The linear slope of the time spent in SWA (Figure 3-7) shows the odds of neurologic mortality increased by 11.0 percent for every month of stay (OR=1.11; 95% CI 1.00, 1.23).

CHAPTER 4: DISCUSSION

This study used the military working dog (MWD) population to evaluate and assess if Gulf War (GW) deployment to Southwest Asia (SWA) was a risk factor for neurologic mortality or peripheral nerve disease (PND).

The study design used variations of the GW MWD cohort to achieve three primary study aims:

(1) Determine the neurologic mortality incidence of the complete GW MWD cohort and the association of neurologic mortality with SWA deployment, and possible confounding variables of age, breed, gender, and branch of military Service.

(2) Determine the PND incidence of the 1997 prospective GW MWD cohort and the association with SWA deployment, and possible confounding variables of age, breed, gender, Service, occupation, and hypothyroid status.

(3) Determine the neurologic mortality association with the SWA deployed MWD by the level of exposure as defined by deployment location, arrival and departure periods, and length of deployment.

The proportion of neurologic mortality cases in the full and prospective GW MWD cohorts of 10.6 percent and 12.0 percent, respectively, was similar to the frequencies reported by Moore et al. [61]. Defined neurologic mortality incidence for the GW cohort was 1.9 cases per 1,000 dog-months, with rates among dogs deployed to SWA elevated above the rates for U.S. and overseas dogs. Analysis of other exposures showed the highest neurologic mortality among dogs deployed to SWA countries other than Saudi Arabia, dogs arriving before or departing after the war, dogs that arrived before and departed after the war, and

dogs that spent more than 176 days in SWA. Peripheral nerve disease incidence was 3.7 cases per 1,000 dog-months for the prospective GW cohort with the rate for the U.S. dogs higher than for overseas and SWA deployed dogs.

Stratified Cox regression was used to adjust for other demographic factors. Adjusted neurologic mortality and PND rates were similar between the U.S., overseas, and SWA assignment locations. Adjusted neurologic mortality and PND rates for periods of arrival and departure and for the number of periods spent in SWA became similar to the non-deployed group. Using logistic regression, an increasing trend in the odds of neurologic mortality was evident with increased time spent in SWA.

Study Strengths

This study is the first known study focusing on neurologic disease among a large MWD population. Comparable neurologic disease measures in other working dog populations are absent from the literature; few studies have reported data on prevalence or incidence. Thus, this study provides invaluable neurologic mortality rates and PND incidence that are characterized by demographic characteristics and geographic and time exposures.

Special care was taken in this study to reduce bias. These actions included masking (blinding) those who performed necropsies, reviewed records, and performed laboratory, pathologic, and nerve conduction analyses. Additional care was taken during the prospective cohort follow-up to emphasize the standardization of care and completion of end of life testing, disposition physicals, and necropsies. Such measures support and strengthen data validity and reduce bias.

All the members of the cohort were accounted for and were included in the study as far as the information allows. Having full enumeration of the cohort reduces any tendency of sampling error. Finally, the observational cohort study design and the data sets formed in this project allow for exploration of associations between other disease outcomes and SWA deployment, or other exposure events.

Study Limitations

Incomplete identification of study eligible members at the time of death reduced the number of nerve pathology samples and end of life laboratory test results. This challenge in the follow-up of study participants introduces the possibility of unforeseen bias in the study. The group with nerve samples was similar to the group not sampled except for a higher proportion of exposed animals and a difference in Service distribution, primarily among U.S. Army and U.S. Navy dogs. The overall similarity in the groups with and without nerve samples adds some confidence that the sampled group was random and representative of the whole prospective cohort.

Even with all the planning to reduce bias, there were some events that cannot be reduced in a working dog population. The dog handlers and treating military veterinarians involved in the health care and disposition decisions of the dogs knew the work history and thus, exposure status of SWA deployed dogs. This information could influence the treatment and longevity of the exposed dogs. This weakness of the study could not be eliminated or controlled for in the analysis. It was assumed that the dogs were handled the same whether exposed or unexposed. There were no striking differences in the measures of association between exposed and unexposed dogs.

All dogs before they deploy are given a physical exam. The animal must be healthy to deploy. Thus, this may cause a selection bias where only healthy dogs deploy and they may be different from those that did not deploy. A similar comparison can be made with the deployability of the military member. If this 'healthy dog effect' were influential, a protective result would be expected to occur for the deployed dogs, which was not seen. The deployability or predeployment health status of the animal, if available, may have been used to control for this effect.

Finally, with this particular population, there was an inability to quantify the amount of exposure to dips, sprays, and other insecticides for flea, tick, and fly repellents that each animal received during their lifetime to control for the possible effect on PND. Even though, the association of pesticide exposure and chronic progressive PND has been reported in cats, the condition has not been reported in dogs [85]. This study assumed that all MWD in the cohort were exposed to pesticides throughout their lifetime at relatively the same levels, internally controlling for the effect of pesticides in this study.

Interpretation

Neurologic mortality has been reported as the third most frequent cause of death/euthanasia in U.S. MWD [61]; for this study cohort it was the fourth most frequent cause. The incidence of neurologic mortality and PND of 1.9 and 3.7 cases per 1,000 dog-months, respectively, can be generalized to the MWD population. At these rates, with a population of 2,000 dogs we could expect 46 dogs a year to die of neurologic problems (13.0 to 18.0% of all deaths per year) and 89 dogs per year to have PND at death (25.0 to 36.0% of all deaths per year).

The high incidence of PND was surprising. Many of these animals (79.0 percent) did not have a clinical neurologic diagnosis and likely demonstrated minimal symptoms. They most likely died or were euthanized for other reasons. Such evidence supports the difficulty and complexity of separating PND from existing mixed conditions. Nerve degeneration and disease appear to occur much more readily than the final disposition physical portrays prior to the death of the animal and more than was evident from the outward symptoms and clinical signs recorded in the medical record of the animal. Knowing that primary neurologic disease in these breeds are demonstrations of chronic processes, the prevalence of PND probably is much higher than previously reported [53, 56, 61, 103].

The relationship between neurologic mortality and PND with SWA deployment was not significantly different from the experience of the dogs in the U.S. or overseas. This finding differed from the results of the 1996 preliminary study. Since 1996, the death categories used to identify the reason of death have been changed and standardized [61]. Additional categories were added to differentiate between orthopedic degenerative diseases, old age or geriatric reasons, and neurologic mortality.

The two study designs were different: the earlier study was cross-sectional; the present one a cohort study. The earlier study measures prevalence, while this study measures incidence. No adjustment was done in the former study to account for the effect of confounding factors.

Finally, the inclusion and exposure times were different. The earlier study included dogs that had died prior to the onset of the GW conflict and the exposure period ended in July rather than December 1991. The current study was able to account for and use all the

participants of the cohort. Overall, the current study allowed for a more realistic portrayal of the neurologic mortality and PND experience in the full cohort.

The lack of an association between SWA exposure and neurologic mortality and PND was not surprising since 91.0 percent of these dogs had deployed from CONUS home stations and the remaining 9.0 percent from Europe. Finding no relationship between SWA and PND confirms similar reports in the human literature [32-34], where GW veterans were not different from those military members who did not deploy.

The elevated rate for dogs deployed to SWA countries other than Saudi Arabia is comparable to Steele's study, which reported the highest prevalence of post-war illness among GW veterans who had served in SWA countries other than Saudi Arabia [2]. The small number of dogs assigned to those areas may have influenced these rates.

Additional similarities to the findings of human studies include the increasing rate of reported illness over departure periods. Both Steele and Spencer et al. reported that illness prevalence patterns in GW veterans were least if departure occurred before the war and were greatest if departure occurred after the war [2, 13]. Steele also reported an increasing trend related to the length of stay, but after adjustment, the experience was similar to that of the comparison group. The trend of increasing neurologic mortality with increasing length of stay within the deployed dogs may need to be examined further. Although the highest quartile showed a tendency toward significance, this does not establish a cause and effect relationship.

Many GW veterans express concern that their illnesses are due to exposures that occurred during their SWA deployment. For geographic location to be an important risk factor for neurologic disease, an environmental exposure to a specific agent that can cause

such an event would need to occur. Such an exposure would need to affect the biologic mechanisms of the nervous system, which would result in the expression of nerve degeneration above normal levels after several years. Southwest Asia is a big place and the likelihood is small that all of the dogs could be exposed specifically to one or more agents capable of producing such effects. Moreover, these dogs were exposed constantly to potential toxic nerve agents throughout their lives. Because of the regular treatment with pesticides, the dogs in this study were reasonably matched internally on pesticide exposure. Any other stimulation of the physiologic and neurologic system by additional agents was not evident from the results of this study.

The effects seen in this study are probably influenced by the demographic factors of age and breed. Age was a confounder in this study. The influencing nature of age has been reported in dogs and humans. Braund et al. has shown that as the dog increases in age, there is a corresponding normal degeneration that takes place in the nerve [68, 69]. Similar studies have been reported in normal elderly persons [40]. This normal nerve degeneration may not affect the MWD usability. The current study demonstrated that MWD with neurologic diseases were older on average than the dogs without the condition. Similar slowly progressive conditions (like degenerative joint disease) in the MWD have been shown not to affect the usability of the animal [61, 112].

The current MWD cohorts had a longer lifespan than dogs in previous MWD studies of the Vietnam-era [57, 59] and pre-GW-era [60]. The longevity of the GW cohort is comparable to another study that includes individuals of this cohort [61]. The higher ages for the MWD may indicate an increase in longevity of the MWD population as a whole and may

be important in understanding the causes of mortality from chronic degenerative problems such as PND, musculoskeletal diseases, and other geriatric conditions.

The results of this study show that the effect of breed on the exposure-outcome relationship was very small. However, the differences found between the breeds confirm an earlier report. Compared to BSD, the GSD had an increased neurologic mortality and PND rates that were similar to the odds ratio reported by Moore et al [61]. GSD have been noted for locomotor problems [53, 55, 60, 112] including chronic progressive posterior paresis, and lumbosacral joint degeneration and instability that could affect the lower lumbar spinal nerves, nerve roots, and the cauda equina. The sloped pelvic conformation and hind limb posture of the GSD has been theorized to predispose the breed to degenerative changes of the posterior axial skeleton and lumbosacral region compared to other working breeds [113]. The heavy training and utilization, including attacking, jumping, unique searching positions, and sudden movements, add additional stress on the lumbosacral junction leading to instability, degeneration, and possible neurologic involvement in these dogs. Trauma from these chronic orthopedic changes may stimulate neuropathic changes [87]. Differentiation between the resulting neurologic and orthopedic symptoms and signs is challenging. There are few reports on BSD, but they are observed to have similar degenerative joint disease and lumbosacral stenosis outcomes as the GSD at necropsy [61, 112].

Public Health Importance

The primary focus of this study was to see if associations could be found between neurologic disease in the MWD population and their geographic exposure to SWA and compare their experience to what was reported in the human literature. This study did not

demonstrate any significant associations between PND and geographic assignment in SWA. Nor did it provide any additional evidence that neurologic disease may be a unique aspect of the illnesses experienced by GW veterans. It did, however, add support to findings in several human studies. This study found illness experiences in MWD that were similar to those reported in military members. Whether this is real or coincidental will require further research.

The MWD population stands as a sentinel animal model. No other comparative group lives and works in the same military environment as the human service member. Previous reports from the Vietnam War era show paired sample increases in titers to tropical diseases between the MWD and their handlers [44]. Another study of mesothelioma in pet dogs, found an association between disease and the occupation of the pet's owner [43]. These findings support the MWD as a sentinel animal.

The MWD with its similar exposure risk, but shorter lifespan, can provide insight on unforeseen risks, evidence for risk assessment, and support cause and effect relationships [15]. Early identification of associations between disease and exposure would allow health care providers to follow exposed humans for illness onset or offer earlier clinical intervention. Although this study did not lead to a sentinel result, it does provide a foundation for other studies that can be done to look for association of other outcomes with GW deployment and other future wartime operations.

Future Directions

The results of this study suggest that deployment to SWA was not associated with the neurological mortality and PND in the MWD. This study was not designed to implicate

any specific toxicologic exposure to chemical or other environmental toxicants. To further explore the sentinel qualities of the MWD population, investigators will need to identify specific toxicologic agents in the work environment that can be measured in both dog and man [15].

It would be useful, then, to look for biomarkers to these environmental toxicants in archived serum and tissues of the prospective cohort. Additional projects could compare biomarker levels with archived information on the environmental air, water, and soil levels for those toxicants at specific locations as well as with the PND or neurologic mortality or other outcomes.

Further studies with MWD in future military deployments could use dogs and their human handlers as paired groups, prospectively collecting blood and urine samples to assess toxic chemical levels in the samples of the dogs and their human handlers. Geo-spatial environmental air, water, and soil data could be collected to compare and correlate location with paired biomarkers for the respective toxic agents of the handler and dog. Such a study would be important in defining the true sentinel relationship between the MWD, the human, and their environment.

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TABLES

Table 2-1. Gulf War military working dog neurologic disease study size requirements for detecting hazard ratios (HR; unexposed/exposed); assumptions: Continental U.S. plus outside continental U.S. locations = unexposed and Southwest Asia = exposed, $\alpha = 0.05$, $1-\beta = 0.80$, estimated median survival time = 100 months, follow-up time = 131 months (17 months recruitment + 114 months additional follow-up).

Available Population	**Study Size Needed**		
Gulf War Cohort	**HR**	**Unexposed**	**Exposed**
Unexposed : Exposed	1.6	1252	79
1997 : 126	1.5	1633	103
15.85 : 1	1.4	2298	145
	0.8	4359	275
	0.7	1649	104
	0.6	777	49
Prospective Cohort (eligible)	**HR**	**Unexposed**	**Exposed**
Unexposed : Exposed	2.2	518	33
612 : 39	2.1	565	36
15.69 : 1	2	628	40
	0.6	769	49
	0.5	408	26
	0.4	220	14
Prospective Cohort (actual)	**HR**	**Unexposed**	**Exposed**
Unexposed : Exposed	2.3	277	31
312 : 35	2.2	303	34
8.91 : 1	2.1	338	38
	0.6	455	51
	0.5	241	27
	0.4	134	15

Table 3-1. Distribution of demographic attributes of the Gulf War military working dog cohort by deployment status

Characteristics	Non-Deployed				Deployed				
	CONUS		OCONUS		SWA		Total		
	n	%	n	%	n	%	n	%	p-value*
Total	957	(45.1)	1,040	(49.0)	126	(5.9)	2,123	(100.0)	
Breed									
Belgian Shepherd	552	(57.7)	601	(57.8)	77	(61.1)	1,230	(57.9)	0.081
German Shepherd	344	(36.0)	340	(32.7)	39	(31.0)	723	(34.1)	
Other	61	(6.4)	99	(9.5)	10	(7.9)	170	(8.0)	
Gender									
Female, spayed	148	(15.5)	146	(14.0)	21	(16.7)	315	(14.8)	0.015
Male, neutered	276	(28.8)	246	(23.7)	40	(31.8)	562	(26.5)	
Male, intact	533	(55.7)	648	(62.3)	65	(51.6)	1,246	(58.7)	
Branch of Service									
Air Force	430	(44.9)	479	(46.1)	109	(86.5)	1,018	(48.0)	<0.001
Army	283	(29.6)	328	(31.5)	6	(4.76)	617	(29.1)	
Navy	166	(17.4)	202	(19.4)	1	(0.8)	369	(17.4)	
Marines	78	(8.2)	31	(3.0)	10	(7.94)	119	(5.6)	

*Pearson's Chi-square

CONUS: Continental United States; OCONUS: Outside Continental United States; SWA: Southwest Asia

Table 3-2. Baseline age and mean age of death (months) in the Gulf War military working dog cohort by breed, gender, Service, and assignment location

Characteristics	Mean Entry Age (mo.)	Mean Death Age (mo.)
Full Gulf War Cohort	69.1	125.0
Range (mo.)	(18, 167)	(25, 178)
Breed		
Belgian Shepherd	57.4	124.9
German Shepherd	86.0	124.0
Other	81.4	129.7
Gender		
Female, spayed	68.8	126.8
Male, neutered	60.2	134.2
Male, intact	73.1	120.3
Branch of Service		
Air Force	71.2	125.5
Army	70.6	125.1
Navy	59.1	121.7
Marines	73.4	130.0
Assignment Location		
Continental United States	65.9	126.6
Outside Continental U.S.	71.8	122.6
Southwest Asia	70.5	132.4

Table 3-3. Distribution of breed, gender, Service, and assignment location of the Gulf War military working dog cohort by neurologic mortality

Characteristics	Cases		Non-cases		
	n	%	n	%	p-value*
Total	226	(10.6)	1,897	(89.4)	
Breed					
Belgian Shepherd	125	(55.3)	1,105	(58.3)	0.095
German Shepherd	89	(39.4)	634	(33.4)	
Other	12	(5.3)	158	(8.3)	
Gender					
Female, spayed	30	(13.3)	285	(15.0)	0.015
Male, neutered	78	(34.5)	484	(25.5)	
Male, intact	118	(52.2)	1,128	(59.5)	
Branch of Service					
Air Force	108	(47.8)	910	(48.0)	0.783
Army	64	(28.3)	553	(29.2)	
Navy	38	(16.8)	331	(17.5)	
Marines	16	(7.1)	103	(5.4)	
Assignment Location					
Continental United States	106	(46.9)	851	(44.9)	0.154
Outside Continental U.S.	101	(44.7)	939	(49.5)	
Southwest Asia	19	(8.4)	107	(5.6)	
Mean Entry Age (mo.)	69.6		69.0		0.776
Mean Death Age (mo.)	131		124.3		<0.001

*Pearson's Chi-square for categorical factors; Students T-Test for means.

Table 3-4. Neurologic mortality rates (cases/1,000 dog-months) and unadjusted rate ratios of the Gulf War military working dog cohort by breed, gender, Service, and assignment location

Characteristics	Cases	Rate (Cases/1,000 Dog-Months)	Rate Ratio (unadjusted)	95% Confidence Interval
Total	226	1.90		
Breed				
Belgian Shepherds	125	1.51	1.00	
German Shepherds	89	3.25	2.16	1.62, 2.85
Other	12	1.46	0.97	0.49, 1.76
Gender				
Female, spayed	30	1.64	1.00	
Male, neutered	78	1.88	1.14	0.74, 1.80
Male, intact	118	2.01	1.22	0.81, 1.89
Branch of Service				
Air Force	108	1.96	1.00	
Army	64	1.90	0.97	0.70, 1.34
Navy	38	1.65	0.84	0.57, 1.23
Marine	16	2.38	1.21	0.67, 2.06
Assignment Location				
Continental United States	106	1.83	1.00	
Outside Continental U.S.	101	1.91	1.05	0.79, 1.39
Southwest Asia	19	2.44	1.33	0.77, 2.19

Table 3-5. Neurologic mortality adjusted rate ratios of the Gulf War military working dog cohort by breed, gender, Service, and age

Characteristics	Rate Ratios (adjusted)*	95% Confidence Interval
Breed		
Belgian Shepherd	1.00	
German Shepherd	2.11	1.55, 2.88
Other	0.74	0.41, 1.36
Gender		
Female, spayed	1.00	
Male, neutered	1.05	0.69, 1.61
Male, intact	1.35	0.90, 2.04
Branch of Service		
Air Force	1.00	
Army	0.96	0.70, 1.33
Navy	1.08	0.74, 1.59
Marine	1.05	0.62, 1.78
Entry Age	1.05	1.04, 1.06

*Cox regression; breed is adjusted for assignment location, gender, Service, and age; all others are stratified by breed and adjusted for assignment location and the other remaining variables

Table 3-6. Neurologic mortality adjusted rate ratios in the Gulf War military working dog cohort by exposure location

	Rate Ratios (adjusted)*	95% Confidence Interval
Deployment Location		
CONUS + OCONUS	1.00	
Southwest Asia	1.00	0.61, 1.63
Assignment Location		
Continental United States	1.00	
Outside Continental U.S.	1.08	0.82, 1.43
Southwest Asia	1.04	0.63, 1.72
Regional Location		
Continental United States	1.00	
Europe	1.18	0.81, 1.71
Pacific	1.09	0.77, 1.53
Americas	0.81	0.42, 1.56
Southwest Asia	1.04	0.63, 1.73

*Cox regression; stratified by breed and adjusted for gender, Service, and age

CONUS: Continental United States; OCONUS: Outside Continental United States

Table 3-7. Distribution of demographic attributes of the 1997 Prospective Gulf War military working dog cohort by deployment status

Characteristics	Non-Deployed				Deployed		Total		
	CONUS		OCONUS		SWA				
	n	%	n	%	n	%	n	%	p-value*
Total	350	(53.8)	262	(40.3)	39	(6.0)	651	(100.0)	
Breed									
Belgian Shepherd	271	(77.4)	211	(80.5)	32	(82.1)	514	(79.0)	0.440
German Shepherd	57	(16.3)	31	(11.8)	6	(15.4)	94	(14.4)	
Other	22	(6.3)	20	(7.6)	1	(2.6)	43	(6.6)	
Gender									
Female, spayed	57	(16.3)	36	(13.7)	8	(20.5)	101	(15.5)	0.633
Male, neutered	153	(43.7)	116	(44.3)	19	(48.7)	288	(44.2)	
Male, intact	140	(40.0)	110	(42.0)	12	(30.8)	262	(40.3)	
Branch of Service									
Air Force	165	(47.1)	98	(37.4)	30	(76.9)	293	(45.0)	<0.001
Army	96	(27.4)	75	(28.6)	1	(2.6)	172	(26.4)	
Navy	76	(21.7)	78	(29.8)	1	(2.6)	155	(23.8)	
Marines	13	(3.7)	11	(4.2)	7	(18.0)	31	(4.8)	
Occupation[#]									
Patrol	109	(32.4)	147	(57.2)	12	(30.8)	268	(42.4)	<0.001
Patrol/Drug	160	(47.6)	70	(27.2)	14	(35.9)	244	(38.6)	
Patrol/Explosive	67	(19.9)	40	(15.6)	13	(33.3)	120	(19.0)	

*Pearson's Chi-square

[#]Occupation information missing for one dog, 17 dogs were drug detectors only, and one dog was a explosive detector only

CONUS: Continental United States; OCONUS: Outside Continental United States; SWA: Southwest Asia

Table 3-8. Baseline age and mean age of death (months) of the 1997 Prospective Gulf War military working dog cohort by breed, gender, Service, and assignment location

Characteristics	Mean Entry Age (mo.)	Mean Death Age (mo.)
Prospective Gulf War Cohort	41.5	139.8
Range (mo.)	(18, 91)	(91, 178)
Breed		
Belgian Shepherd	41.6	140.3
German Shepherd	41.9	136.3
Other	39.7	140.6
Gender		
Female, spayed	42.0	142.2
Male, neutered	41.9	140.9
Male, intact	40.8	137.5
Branch of Service		
Air Force	41.0	139.2
Army	42.3	140.5
Navy	41.0	140.5
Marines	44.4	142.8
Assignment Location		
Continental United States	39.4	139.0
Outside Continental U.S.	43.6	140.2
Southwest Asia	46.0	143.6

Table 3-9. Neurologic mortality adjusted rates ratios of the 1997 Prospective Gulf War military working dog cohort by demographic variables and assignment location (n=651)

Characteristics	Cases	Rate Ratio (adjusted)*	95% Confidence Interval
Total	78		
Breed			
Belgian Shepherds	64	1.00	
German Shepherds	10	1.08	0.54, 2.13
Other	4	0.75	0.27, 2.12
Gender			
Female, spayed	11	1.00	
Male, neutered	38	1.35	0.68, 2.69
Male, intact	29	1.51	0.74, 3.09
Branch of Service			
Air Force	36	1.00	
Army	15	0.70	0.37, 1.30
Navy	24	1.45	0.84, 2.49
Marine	3	0.62	0.19, 2.03
Assignment Location			
Continental United States	44	1.00	
Outside Continental U.S.	28	0.81	0.43, 2.55
Southwest Asia	6	1.05	0.49, 1.32
Age		1.07	1.05, 1.09

*Cox regression; breed is adjusted for assignment location, gender, Service, and age; all others are stratified by breed and adjusted for assignment location and the other remaining factors

Table 3-10. Distribution of demographic attributes and assignment location in the 1997 Prospective military working dog cohort by nerve pathology sample status

Characteristics	With Samples		No Samples		
	n	%	n	%	p-value*
Total	347	(53.3)	304	(46.7)	
Breed					
Belgian Shepherd	278	(80.1)	236	(77.6)	0.169
German Shepherd	52	(15.0)	42	(13.8)	
Other	17	(4.9)	26	(8.6)	
Gender					
Female, spayed	57	(16.4)	44	(14.5)	0.614
Male, neutered	156	(45.0)	132	(43.4)	
Male, intact	134	(38.6)	128	(42.1)	
Branch of Service					
Air Force	159	(45.8)	134	(44.1)	0.018
Army	77	(22.2)	95	(31.3)	
Navy	96	(27.7)	59	(19.4)	
Marines	15	(4.3)	16	(5.3)	
Occupation[#]					
Patrol	152	(45.0)	116	(39.5)	0.314
Patrol/Drug	122	(36.1)	122	(41.5)	
Patrol/Explosive	64	(18.9)	56	(19.1)	
Assignment Location					
Continental United States	195	(56.2)	155	(51.0)	<0.001
Outside Continental U.S.	117	(33.7)	145	(47.7)	
Southwest Asia	35	(10.1)	4	(1.3)	
Mean Entry Age (mo.)	44.6		37.9		<0.001
Mean Death Age (mo.)	139.1		140.5		0.265

*Pearson's Chi-square for categorical factors; Students T-Test for means

[#]Missing occupation or single discipline detectors: nine dogs for those sampled and ten dogs for those not sampled

Table 3-11. Distribution of demographic attributes and assignment location of the 1997 Prospective military working dog cohort with nerve pathology samples by peripheral nerve disease

Characteristics	Cases		Non-Cases		
	n	%	n	%	p-value*
Total	121	(34.9)	226	(65.1)	
Breed					
Belgian Shepherd	100	(82.6)	178	(78.8)	0.685
German Shepherd	16	(13.2)	36	(15.9)	
Other	5	(4.1)	12	(5.3)	
Gender					
Female, spayed	26	(21.5)	31	(13.7)	0.061
Male, neutered	57	(47.1)	99	(43.8)	
Male, intact	38	(31.4)	96	(42.5)	
Branch of Service					
Air Force	52	(43.0)	107	(47.4)	0.060
Army	32	(26.5)	45	(19.9)	
Navy	28	(23.1)	68	(30.1)	
Marines	9	(7.4)	6	(2.7)	
Occupation[#]					
Patrol	50	(43.1)	102	(46.0)	0.860
Patrol/Drug	44	(37.9)	78	(35.1)	
Patrol/Explosive	22	(19.0)	42	(18.9)	
Assignment Location					
Continental United States	72	(59.5)	123	(54.4)	0.657
Outside Continental U.S.	38	(9.1)	24	(10.6)	
Southwest Asia	11	(31.4)	79	(35.0)	
Mean Entry Age (mo.)	45.6		44.0		0.311
Mean Death Age (mo.)	145.8		135.5		<0.001

*Pearson's Chi-square for categorical factors; Students T-Test for means

[#]Missing occupation or single discipline detectors: nine dogs for those sampled

Table 3-12. Peripheral nerve disease rates (cases/1,000 dog-months) and unadjusted rate ratios of the 1997 Prospective Gulf War military working dog cohort with nerve pathology samples by demographic variables and assignment location

Characteristics	Cases	Rate (Cases/1,000 Dog-Months)	Rate Ratio (unadjusted)	95% Confidence Interval
N=347	121	3.69		
Breed				
Belgian Shepherds	100	3.78	1.00	
German Shepherds	16	3.36	0.89	0.49, 1.52
Other	5	3.13	0.83	0.26, 2.00
Gender				
Female, spayed	26	4.75	1.00	
Male, neutered	57	3.84	0.81	0.50, 1.34
Male, intact	38	3.04	0.64	0.38, 1.10
Branch of Service				
Air Force	52	3.49	1.00	
Army	32	4.34	1.24	0.77, 1.97
Navy	28	3.09	0.88	0.54, 1.43
Marine	9	6.17	1.77	0.77, 3.62
Occupation				
Patrol	50	3.53	1.00	
Patrol/Drug	44	3.73	1.06	0.69, 1.62
Patrol/Explosive	22	3.67	1.04	0.60, 1.75
Assignment Location				
Continental United States	72	3.86	1.00	
Outside Continental U.S.	38	3.53	0.91	0.60, 1.37
Southwest Asia	11	3.21	0.83	0.40, 1.58

[#]Missing occupation or single discipline detectors: nine dogs for those sampled

Table 3-13. Peripheral nerve disease adjusted rate ratios of the 1997 Prospective Gulf War military working dog cohort with nerve pathology samples by demographic variables

Characteristics	Rate Ratios (adjusted)*	95% Confidence Interval
Breed		
Belgian Shepherd	1.00	
German Shepherd	1.70	0.97, 2.96
Other	0.60	0.21, 1.73
Gender		
Female, spayed	1.00	
Male, neutered	0.94	0.54, 1.63
Male, intact	1.35	0.75, 2.42
Branch of Service		
Air Force	1.00	
Army	1.36	0.82, 2.25
Navy	1.12	0.66, 1.93
Marine	1.47	0.66, 3.27
Occupation		
Patrol	1.00	
Patrol/Drug	0.82	0.50, 1.35
Patrol/Explosive	0.81	0.44, 1.51
Entry Age	1.07	1.05, 1.08

*Cox regression; breed is adjusted for assignment location, gender, Service, occupation, hypothyroid status, and age; all others are stratified by breed and adjusted for assignment location and the other remaining factors

Table 3-14. Peripheral nerve disease adjusted rate ratios of the 1997 Prospective Gulf War military working dog cohort with nerve pathology samples by exposure location

	Rate Ratios (adjusted)*	95% Confidence Interval
Deployment Location		
CONUS + OCONUS	1.00	
Southwest Asia	0.98	0.48, 2.02
Assignment Location		
Continental United States	1.00	
Outside Continental U.S.	1.17	0.70, 1.93
Southwest Asia	1.02	0.49, 2.13
Regional Location		
Continental United States	1.00	
Europe	1.46	0.64, 3.31
Pacific	1.39	0.75, 2.57
Americas	0.63	0.24, 1.65
Southwest Asia	1.02	0.49, 2.11

*Cox regression; stratified by breed and adjusted for gender, Service, occupation, hypothyroid status, and age

CONUS: Continental United States; OCONUS: Outside Continental United States

Table 3-15. Distribution of demographic attributes, deployment location, and period of arrival and departure of the Southwest Asia deployed military working dogs by mean deployment length

Characteristics	Frequency	%	Mean Deployment Length (days)
Total	N=126		133.2
Range (days)			(30, 304)
Breed			
Belgian Shepherd	77	(61.1)	130.5
German Shepherd	39	(31.0)	141.9
Other	10	(7.9)	120.0
Gender			
Female, spayed	21	(16.7)	139.4
Male, neutered	40	(31.8)	127.3
Male, intact	65	(51.6)	134.9
Branch of Service			
Air Force	109	(86.5)	135.6
Army	6	(4.8)	128.0
Navy	1	(0.8)	226.0
Marines	10	(7.9)	101.3
Occupation			
Patrol	35	(28.2)	132.3
Patrol/Drug	39	(31.5)	145.1
Patrol/Explosive	50	(40.3)	126.0
Deployment Location			
Saudi Arabia	109	(86.5)	135.9
Other SWA Countries	17	(13.5)	115.8
Arrival Period			
Pre-war	78	(61.9)	153.7
War	40	(31.8)	103.4
Post-war	8	(6.4)	83.3
Departure Period			
Pre-war	3	(2.4)	76.3
War	40	(31.8)	129.5
Post-war	83	(65.9)	137.1

SWA: Southwest Asia

Table 3-16. Baseline age, mean age at death, and deployment length of the Southwest Asia deployed military working dogs by neurologic mortality (n=126)

	Cases	**Non-Cases**	**p-value***
Mean Entry Age (mo.)	73.6	70.0	0.61
Mean Death Age (mo.)	133.6	132.2	0.81
Mean Deployment Length (days)	146.4	130.9	0.24

*Students T-Test for means.

Table 3-17. Neurologic mortality rates (cases/1,000 dog-months), and rate ratios of Southwest Asia deployed military working dogs by breed, gender, Service, and occupation

Characteristics	Cases	Rate (Cases/1,000 Dog-Months)	Rate Ratio (unadjusted)	95% Confidence Intervals
N=126	19	2.44		
Breed				
Belgian Shepherd	11	2.00	1.00	
German Shepherd	6	3.31	1.66	0.50, 4.89
Other	2	4.10	2.05	0.22, 9.39
Gender				
Female, spayed	5	3.70	1.00	
Male, neutered	8	2.65	0.72	0.21, 2.79
Male, intact	6	1.75	0.47	0.12, 1.96
Branch of Service				
Air Force	19	2.90		
Occupation				
Patrol	5	2.38	1.00	
Patrol/Drug	5	1.91	0.80	0.18, 3.48
Patrol/Explosive	9	2.97	1.25	0.37, 4.73

Table 3-18. Neurologic mortality rates (cases/1,000 dog-months), and rate ratios of the Gulf War military working dog cohort by deployment location, arrival and departure period, number of periods and number of days (quartiles) spent in Southwest Asia.

Exposure Factors	Cases	Rate (Cases/1,000 Dog-Months)	Rate Ratio (unadjusted)	95% Confidence Intervals
Reference Category: CONUS + OCONUS (No time in SWA)	207	1.87	1.00*	
Deployment Location				
Saudi Arabia	16	2.35	1.26	0.71, 2.10
Other SWA Countries	3	2.98	1.60	0.32, 4.73
Arrival Period				
Pre-war	14	2.96	1.59	0.85, 2.72
War	5	2.02	1.08	0.34, 2.56
Post-war	0	0	0	--
Departure Period				
Pre-war	0	0	0	--
War	5	2.07	1.10	0.36, 2.62
Post-war	14	2.69	1.44	0.77, 2.47
# of Periods Spent in SWA				
1 Period	0	0	0	--
2 Periods	10	2.43	1.30	0.62, 2.43
3 Periods	9	3.56	1.91	0.86, 3.69
Time Spent in SWA (quartiles)				
30-92 days **(1)**	3	1.47	0.79	0.16, 2.34
93-122 days **(2)**	5	2.30	1.23	0.40, 2.92
124-176 days **(3)**	5	2.69	1.44	0.46, 3.41
>176 days **(4)**	6	3.47	1.86	0.67, 4.11

*Reference category for all comparisons: CONUS + OCONUS, no time spent in SWA

CONUS: Continental United States; OCONUS: Outside Continental United States; SWA: Southwest Asia

Table 3-19. Neurologic mortality adjusted rate ratios of the Gulf War military working dog cohort by deployment location, arrival and departure periods, and number of periods spent in Southwest Asia

Exposure Factors	Rate Ratios (adjusted)*	95% Confidence Intervals
Reference Category: CONUS + OCONUS (No time in SWA)	1.00[#]	
Deployment Location		
Saudi Arabia	0.91	0.54, 1.54
Other SWA Countries	2.14	0.66, 6.89
Arrival Period		
Pre-war	1.14	0.65, 1.99
War	0.94	0.38, 2.31
Post-war	0	--
Departure Period		
Pre-war	0	--
War	0.96	0.39, 2.36
Post-war	1.06	0.60, 1.85
# of Time Periods		
1 Period	0	--
2 Periods	1.13	0.59, 2.16
3 Periods	1.22	0.61, 2.43

*Cox regression; stratified by breed and adjusted for gender, Service, and age
[#]Reference category for all comparisons: CONUS + OCONUS, no time spent in SWA
CONUS: Continental United States; OCONUS: Outside Continental United States; SWA: Southwest Asia

Table 3-20. Neurologic mortality adjusted rate ratios of the Gulf War military working dog cohort by the number of days spent in Southwest Asia (quartiles)

Exposure Factors	Odds Ratios (adjusted)*	95% Confidence Intervals
Reference Category: CONUS + OCONUS (No time in SWA)	1.00[#]	
Time Spent in SWA (quartiles)		
30-92 days **(1)**	0.85	0.25, 2.83
93-122 days **(2)**	1.71	0.64, 4.60
124-176 days **(3)**	1.57	0.59, 4.18
>176 days **(4)**	2.18	0.87, 5.45
Time Spent in SWA (months)	1.11	1.00, 1.23

Trend test: p=0.047

*Logistic regression; adjusted for breed, gender, Service, and age

[#]Reference category for all comparisons: CONUS + OCONUS, no time spent in SWA

CONUS: Continental United States; OCONUS: Outside Continental United States; SWA: Southwest Asia

FIGURES

Figure 2-1: The Gulf War military working dog cohort by Gulf War deployment exposure and time periods.

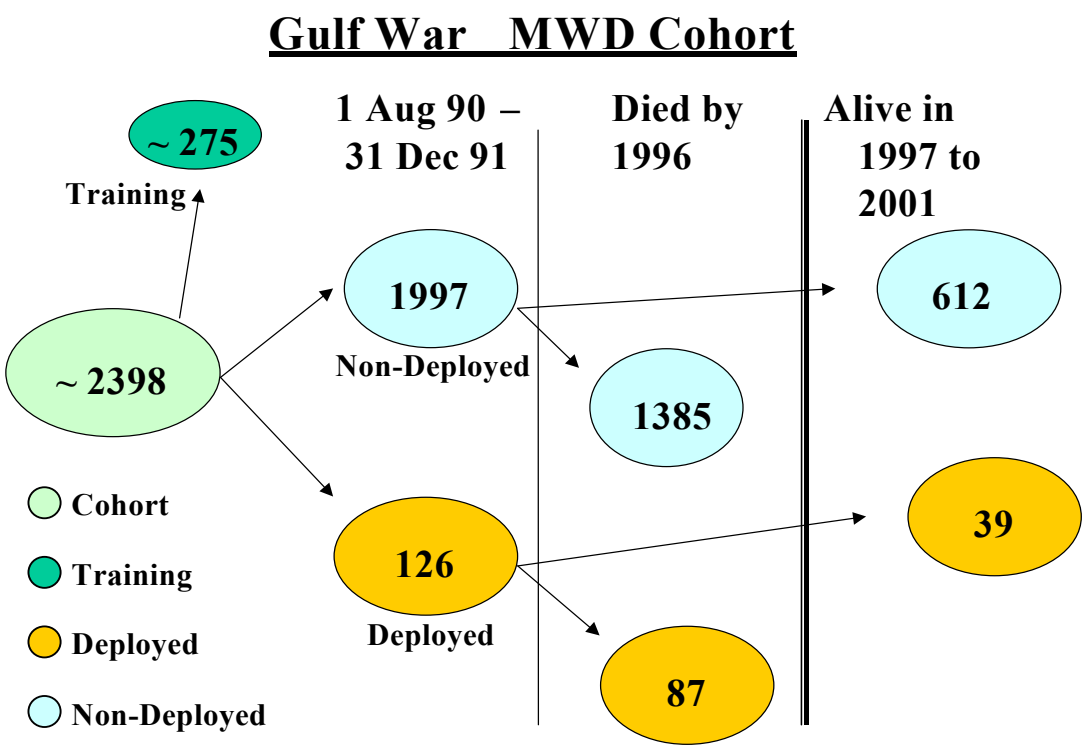


Figure 3-1. Log-Log survival probability plot of neurologic mortality over the followed analysis time (months) for German Shepherd Dogs vs Belgian Shepherd Dogs, unadjusted for other factors.

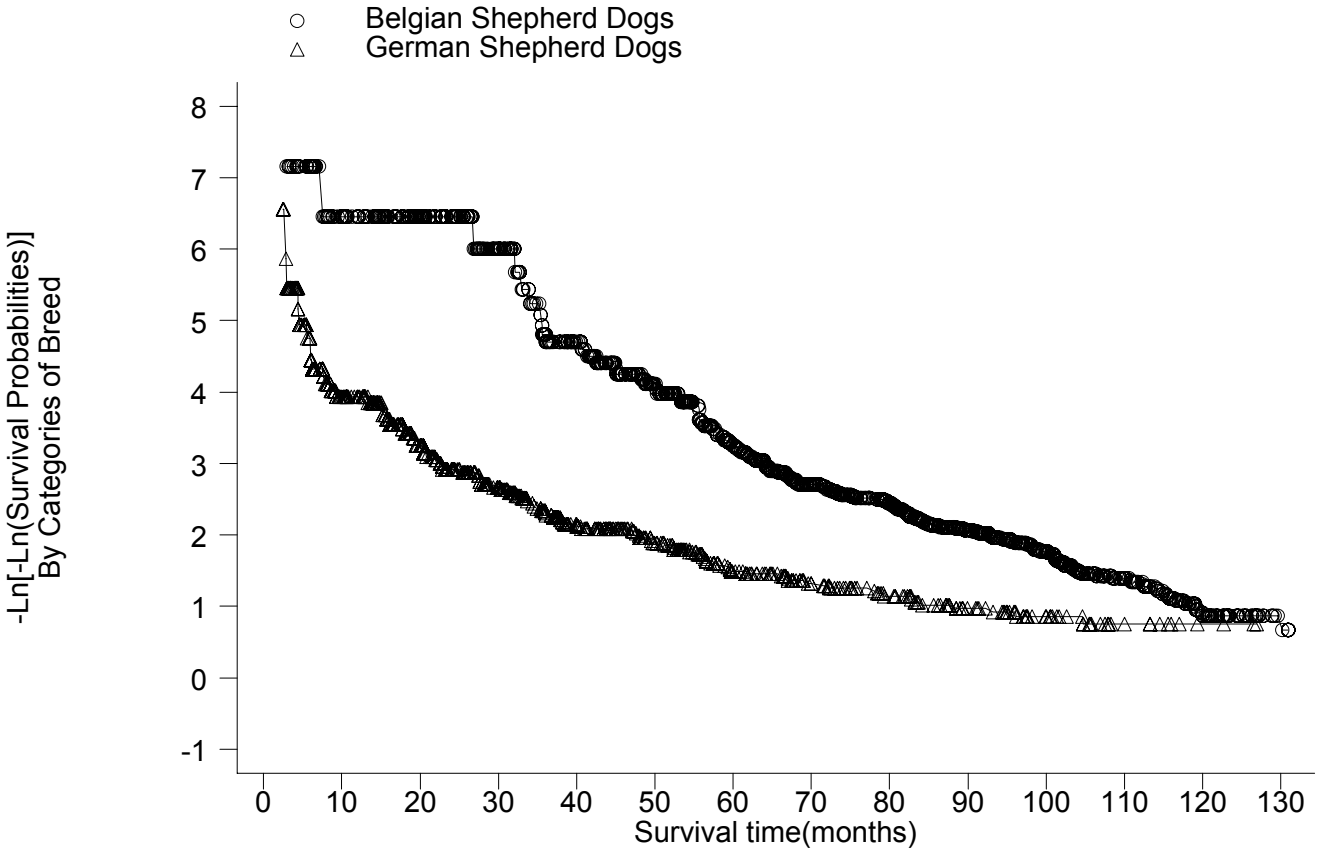


Figure 3-2. Log-Log survival probability plot of neurologic mortality over the followed analysis time (months) for German Shepherd Dogs vs Belgian Shepherd Dogs, adjusted for assignment location, age at entry, branch of Service, and gender.

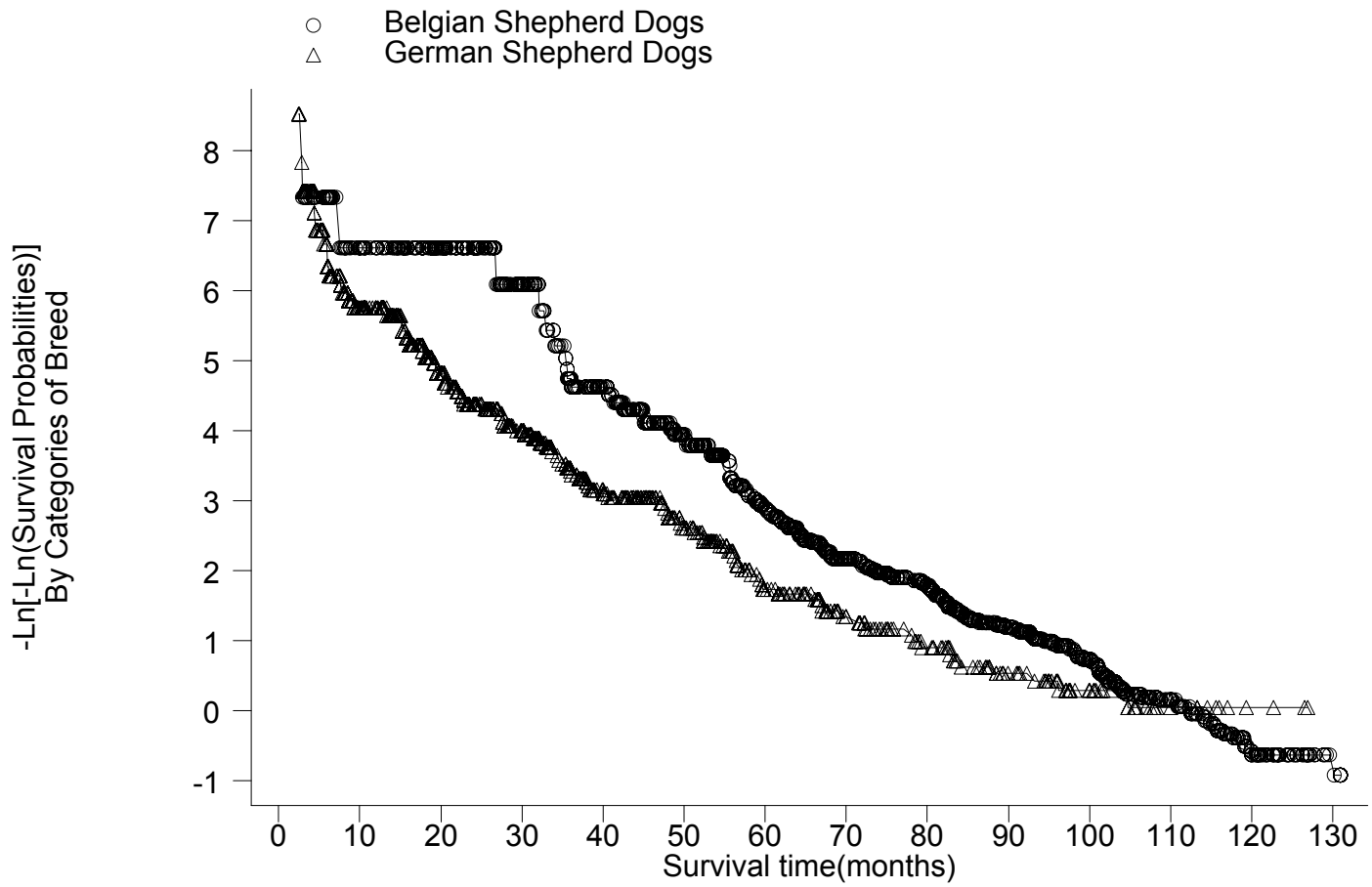


Figure 3-3. Log-Log survival probability plot of peripheral nerve disease over the followed analysis time (months) for German Shepherd Dogs vs Belgian Shepherd Dogs, adjusted for assignment location, age at entry, branch of Service, gender, hypothyroid status, and occupation.

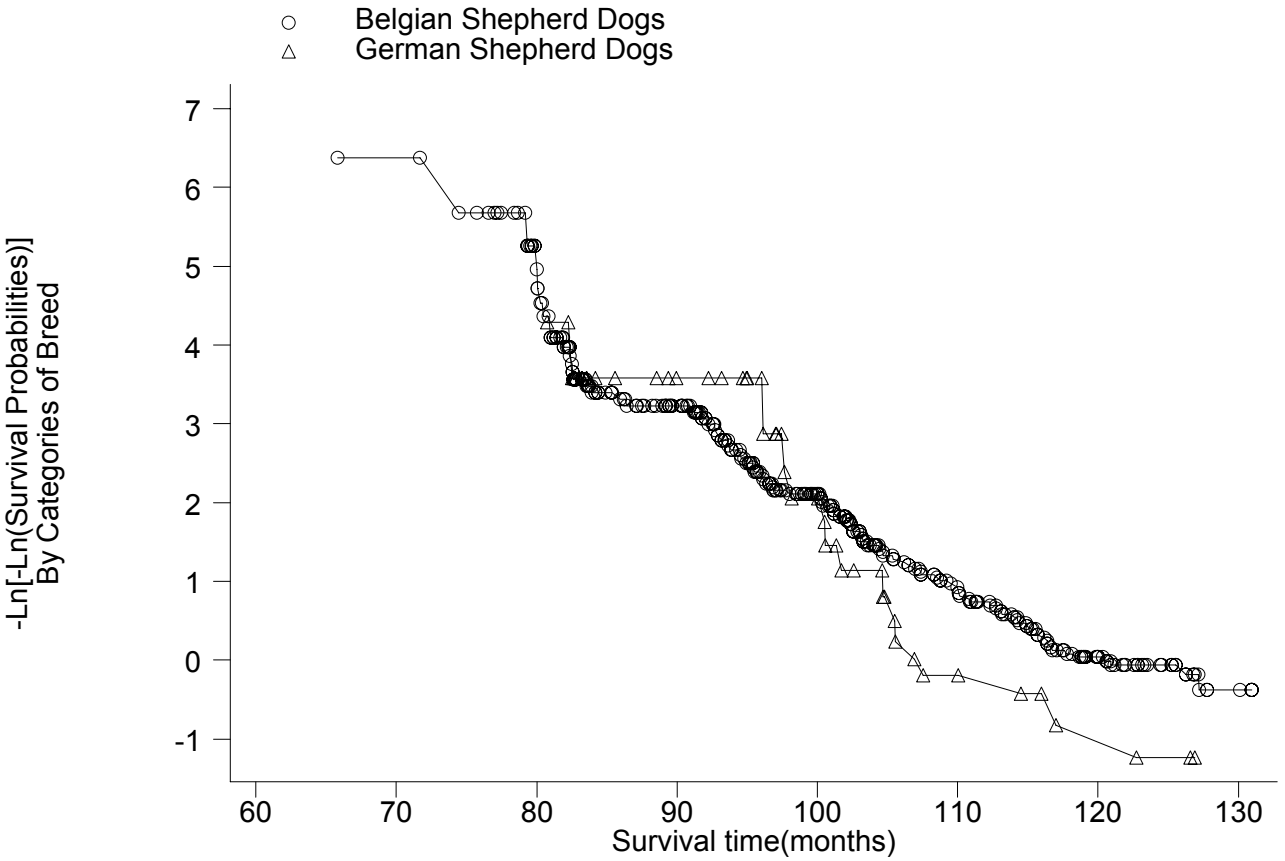
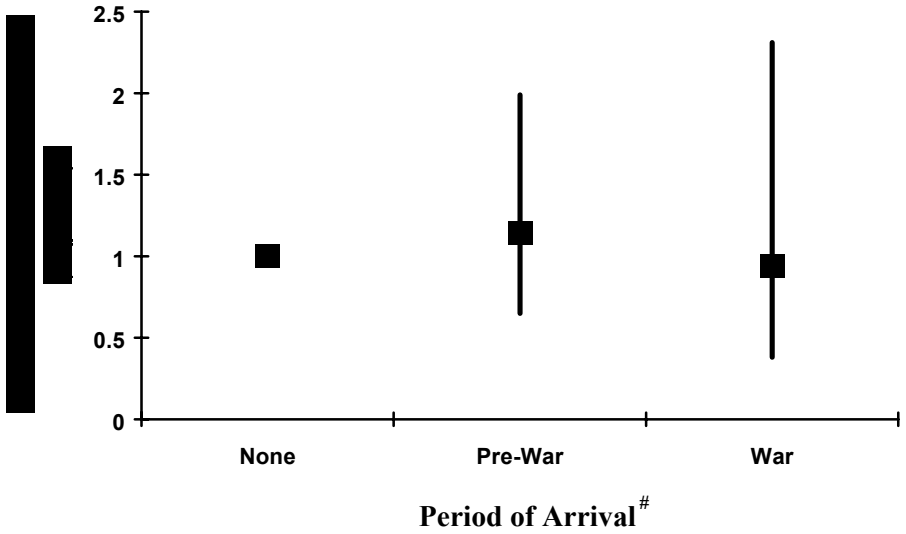
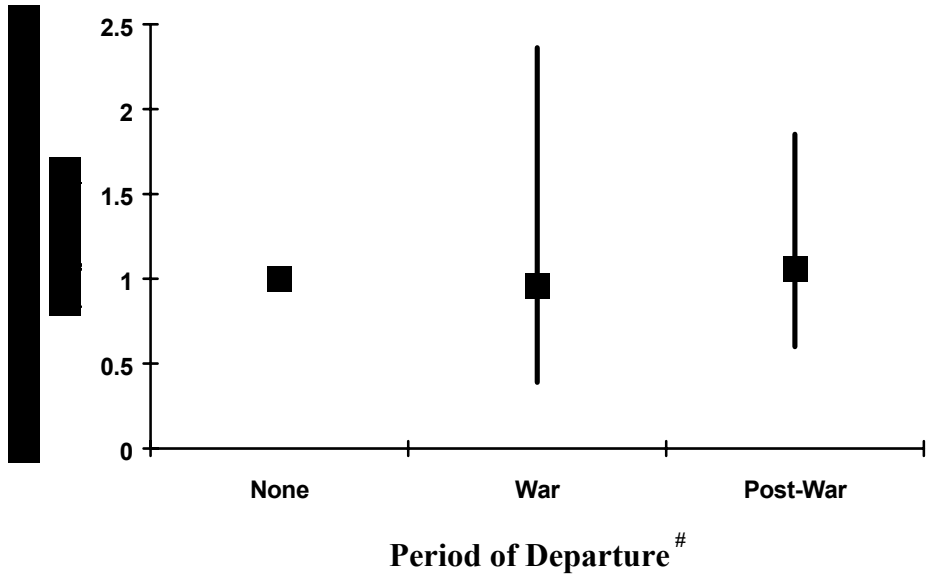


Figure 3-4. Neurologic mortality rate ratios and 95% confidence intervals by period of arrival to Southwest Asia during the Gulf War, 1 August 1990 to 31 December 1991



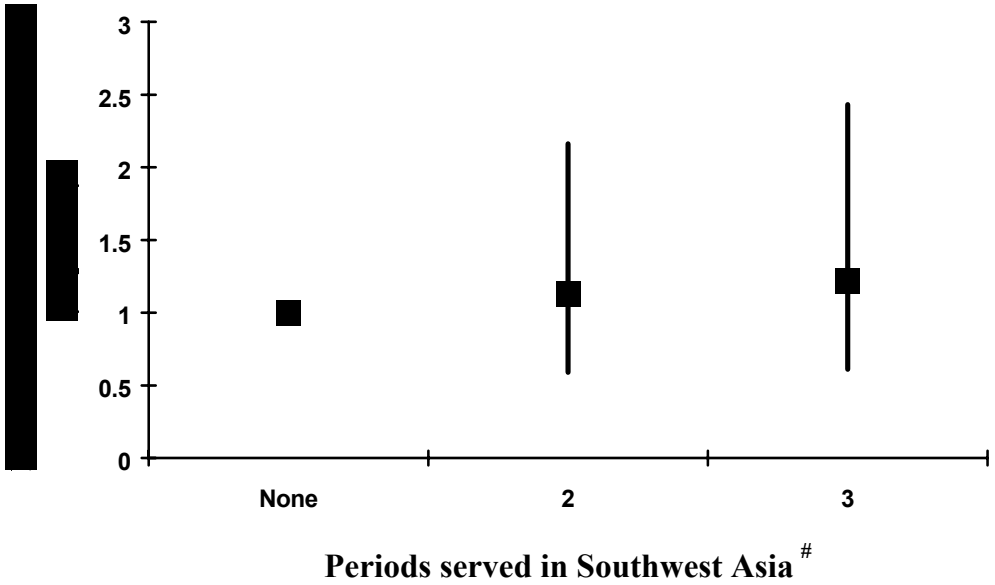
*Cox regression; stratified by breed and adjusted for gender, Service, and age
#No cases in dogs deployed after the war

Figure 3-5. Neurologic mortality rate ratios and 95% confidence intervals by period of departure to Southwest Asia during the Gulf War, 1 August 1990 to 31 December 1991



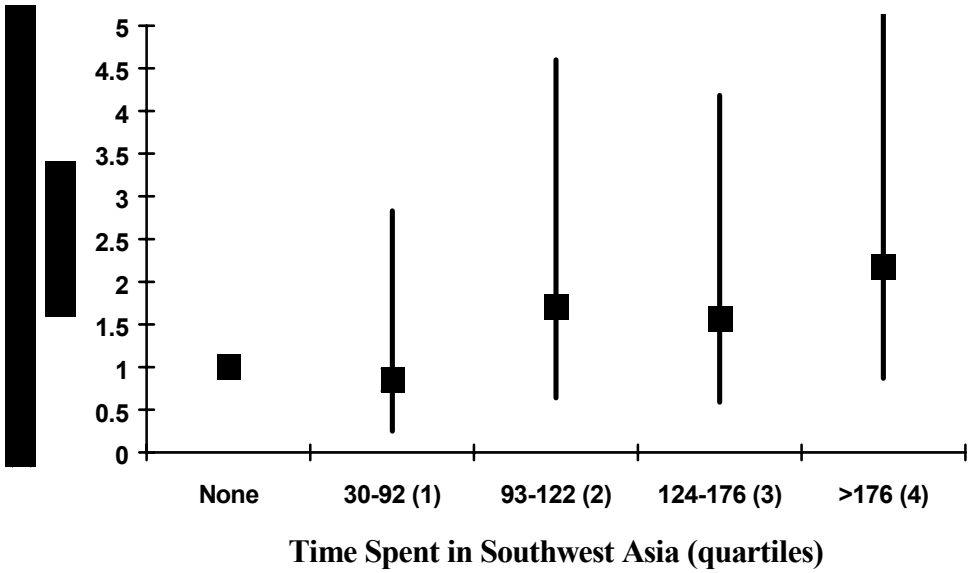
*Cox regression; stratified by breed and adjusted for gender, Service, and age
#No cases in dogs departing before the war

Figure 3-6. Neurologic mortality rate ratios and 95% confidence intervals by the number of periods served in Southwest Asia during the Gulf War, 1 August 1990 to 31 December 1991



*Cox regression; stratified by breed and adjusted for gender, Service, and age
#No cases in dogs that served only one period

Figure 3-7. Neurologic mortality odds ratios and 95% confidence intervals by the number of days spent in Southwest Asia during the Gulf War (quartiles), 1 August 1990 to 31 December 1991



*Logistic regression; adjusted for breed, gender, Service, and age
Trend significance p=0.047

APPENDICES

Appendix A. Acronym/Symbol Definitions

AChE	Acetyl Cholinesterase
AFIP	Armed Forces Institute of Pathology
BSD	Belgian Shepherd Dog
CCEP	Comprehensive Clinical Evaluation Program
CONUS	Continental United States
cTSH	Canine Thyroid Stimulating Hormone; thyrotropin
DHHS	Department of Health and Human Services
DoD	Department of Defense
DODMWDVS	Department of Defense Military Working Dog Veterinary Services
FT4	Serum Free Thyroxine
GSD	German Shepherd Dog
GW	Gulf War
HR	Hazard Ratio
IACUC	Institutional Animal Care and Use Committee
KSA	Kingdom of Saudi Arabia
MWD	Military Working Dog
OCONUS	Outside Continental United States
PB	Pyridostigmine Bromide
PND	Peripheral Nerve Disease
PTSS	Posttraumatic Stress Symptomatology
RR	Rate Ratio
SNOMED® International	Systemized Nomenclature of Medicine for Human and Veterinary Medicine
SWA	Southwest Asia
T4	Thyroxine
TCDD	Tetrachlorodibenzodioxin
U.S.	United States
USA	United States Army
USAF	United States Air Force
USMC	United States Marine Corps
USN	United States Navy
VA	Department of Veterans Affairs

Appendix B. AFIP Protocol UBAD2

"Indicators of Human Disease from Persian Gulf War Service: A Study of Military Working Dogs deployed in Operations Desert Shield/Storm"

-- Protocol Conditional Approval, 18 February 1997
-- IACUC Conditional Approval, 16 June 1997
-- Protocol Final Approval, 5 September 1997
-- IACUC Final Approval, 5 September 1997
-- AFIP Protocol UBAD2: Update, 31October 2000

DEPARTMENT OF THE ARMY
ARMED FORCES INSTITUTE OF PATHOLOGY
WASHINGTON, DC 20306-6000

REPLY TO
ATTENTION OF

18 February 1997

AFIP-RR

MEMORANDUM FOR LTC ROBERT MOELLER, DEPT OF VETERINARY PATHOLOGY

SUBJECT: Conditional Approval of Protocol

1. I have reviewed your protocol, "Indicators of Human Disease from Persian Gulf War Service: A Study of Military Working Dogs Deployed in Operations Desert Shield/Storm:, and grant conditional approval of the protocol for the purpose of submitting it to an outside granting agency. The protocol must still be reviewed at a full meeting of the Research Committee prior to beginning work.

2. If you have any questions concerning this conditional approval, please contact my office at (202) 782-2103 or Ms. Annette Anderson, Administrator, Repository and Research Services, at (202) 782-2500.

GLENN N. WAGNER, CAPT, MC, USN
Chairperson, Research Committee

CF:
A. Anderson

ACTION ON APPLICATION FOR ANIMAL USE

ARMED FORCES INSTITUTE OF PATHOLOGY
LABORATORY ANIMAL CARE AND USE COMMITTEE

1. TITLE OF INVESTIGATION AND NAMES OF INVESTIGATORS: Analysis of Human Disease from Persian gulf War Service: Study of Military Working Dogs Deployed in Operations Desert Shield/Storm, Robert B. Moeller, Jr., LTC, VC, USA

---

2. DECISION OF COMMITTEE MEMBERS:

( ) The use of animals is **APPROVED**. The project will be forwarded to the AFIP Research Committee.

(X) The use of animals is **CONDITIONALLY APPROVED** pending minor changes be made as recommended below and submitted in written form to the AFIP Research Office. The project will be forwarded to the AFIP Research Committee after this documentation has been reviewed and accepted as complete.

( ) The project is **DEFERRED** for revision and resubmission for the reasons indicated below:

( ) The project is **DISAPPROVED** for the reasons indicated below:

REMARKS:

1. Deletion of the paragraph dealing with pain alleviation and anesthesia, analgesia, and tranquilization (V.C.7.b.[1]) since it is an exact duplication of a preceding paragraph dealing with animal manipulations and injections (paragarph V.C.3.a.) and is confusing since all animals are in the no pain category.

2. Signing of the signature block in paragraph VI.G.

Frances E. Moeller
Recorder
Date: 6/16/97

Gary K. Ellis
Chairperson
Date: 6/16/97

AFIP Form 50-9 (Jun 1996)

DEPARTMENT OF THE ARMY
ARMED FORCES INSTITUTE OF PATHOLOGY
WASHINGTON, DC 20306-6000

REPLY TO
ATTENTION OF

5 September 1997

AFIP-RR

MEMORANDUM FOR LTC ROBERT MOELLER, DEPARTMENT OF VETERINARY PATHOLOGY

SUBJECT: Notice of Final Approval of Protocol

1. Your protocol, "Indicators of Human Disease from Persian Gulf War Service: A Study of Military Working Dogs Deployed in Operation Desert Shield/Storm," has received final (unconditional) approval.

2. Since you indicated that this protocol was extension of the protocol "Military Working Dogs Deployed to Southwest Asia as Sentinels for Human Environmental Exposure During the Persian Gulf War: Investigation of the Desert Storm Mystery Disease" which is assigned research identification code UBAD, this protocol will be given a subidentifier of UBAD2. The previous protocol will now be carried in our records as UBAD1.

3. You must use the first four digits of the research identification code when requesting any services in conjunction with this research protocol. Failure to do so could result in inappropriate charges to departmental budgets, an underestimation of the cost of research, and a potential loss of funds to the AFIP.

4. Any questions on this matter should be addressed to the Research Office at 782-2500, Room G061.

Annette R. Anderson

Encl Approval Document
Research ~~Identification Code~~

ANNETTE R. ANDERSON, MS, RRA
Administrator, Repository and Research Services

ACTION ON APPLICATION FOR RESEARCH

ARMED FORCES INSTITUTE OF PATHOLOGY
RESEARCH COMMITTEE

1. PROTOCOL TITLE AND NAME OF AFIP PRINCIPAL INVESTIGATORS: Analysis of Human Disease from Persian Gulf War Service: Study of Military Working Dogs Deployed in Operations Desert Shield/Storm - Robert B. Moeller, Jr., LTC, VC, USA.

2. RECOMMENDATION BY COMMITTEE MEMBERS:

(X) Approval ( ) Disapproval

( ) Deferment for revision/resubmission as specified below:

( ) Conditional Approval pending resolution of the following items:

Annette R. Anderson, 9/4/97
Recorder/Date

G M Wagner 9/5/97
Chairperson/Date

3. DECISION OF DIRECTOR, AFIP:

(✓) Approved

( ) Disapproved

( ) Deferred for revision/resubmission

( ) Conditional Approval pending resolution of items listed above

9/5/97
The Director/Date

AFIP Protocol UBAD2

"Indicators of Human Disease from Persian Gulf War Service: A Study of Military Working Dogs deployed in Operations Desert Shield/Storm,"

Protocol Update, 31October 2000:

Principal Investigator of AFIP Protocol UBAD2:

LTC Dale G. Dunn, VC, USA
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AMEDD Personnel Proponency
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Wilford Hall, Lackland AFB, TX
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A. Experimental Design and General Procedures:

MWD receive semi-annual physical examinations throughout their active duty lives, which include clinical evaluations and routine panels of hematologic, serologic, and blood chemical analyses, the results of which are recorded in the medical record. Those MWD identified for this study that are euthanized for medical reasons will have peripheral blood samples collected prior to euthanasia for the above blood tests; results are included in the dog's permanent medical record. Necropsies are performed in accordance with a standard protocol contained in Technical Bulletin-Medical (TB Med) 283. Medical records from all deceased MWD in the Department of Defense are archived at the DODMWDVS, Lackland AB, Texas.

Based on the assumptions of a condition with 10% prevalence in the population, looking for a relative risk of 2.5 in the exposed group, setting the alpha level at 0.05 and the beta level at 0.2, a minimum of 112 animals of each group must be included in the study. Therefore, the medical and training records of those 118 MWD which deployed to the Persian Gulf, and 472 nondeployed MWD matched four to one based on age, gender and breed, will be abstracted during the study period for the following variables: animal identification; age at death; date of death; breed; gender; location during the time frame 1 August 1990 to 31 December 1991; duration of deployment; neurologic illness; orthopedic illness; dermatologic illness; gastrointestinal illness; infectious diseases; parasitisms; neoplasms; behavioral changes after 1 August 1990; pathological diagnoses of biopsy specimens and pathological diagnoses of autopsy specimens. This data will be electronically stored in a database FOXPRO® for statistical analysis using the SPSS® analysis program. The three remaining Persian Gulf exposed MWD will be transported to the DODMWDVS, Lackland AB when the responsible Veterinary Corps Officer has determined the animal is no longer physically fit for duty and is in need of euthanasia. The procedures that follow have already been completed on the respective control animals for these three veteran dogs. The MWD will receive a complete physical exam, to include: CBC/WBC; Serum Chemistry Panel; acetylcholinesterase activity levels; urinalysis; fecal exam for parasites; thyroid hormone measurements; electrocardiography; radiography; a neurologic examination in accordance with the Colorado State University protocol (Tab A, Annex 1) and a behavioral assessment in according with the protocol at Tab A, Annex 2. Radiographs of elbows, stifles, coxofemoral joints and spine will be obtained, if not present in the record.
The dog will then be euthanized with a standard approved injectable euthanasia agent and immediately necropsied in accordance with TB Med 283. Specimens of biceps femoris and triceps brachii muscles, and tibial and radial nerves will be collected for analysis in accordance with the Auburn University protocol at Tab A, Annex 3. Additionally, 6-gram samples of liver, kidney, lung, brain and fat will be collected for ultra low temperature freezing. The brain and the complete spinal cord will be collected for a thorough histopathological analysis of the central nervous system. Formalin fixed tissues are processed for histopathological examination resulting in a detailed final pathology report consisting of a list of pathological findings and an interpretation of these findings. Remaining wet tissues, paraffin blocks, micro slides and case folder materials are archived. The pathology report is forwarded to the DODMWDVS, Lackland AB, TX for inclusion in the MWD' medical record. Frozen samples are transported the US Army Veterinary Laboratory, Fort Sam Houston, TX for ultra low temperature banking.

All data collected during the final examination procedures will be electronically stored in a FOXPRO® database for statistical analysis using the SPSS® statistical program. Upon completion of data collection, a multivariate analysis of collected variables will be accomplished to determine the effects of age, gender and breed on those conditions commonly occurring in the entire population of MWD. Odds ratios and ninety-five percent confidence intervals will be calculated on all conditions occurring more frequently in one cohort to determine the effects of the exposure status on those conditions. Fisher's exact p values will be calculated to determine statistical significance of any conditions occurring more frequently in one cohort.

B. Laboratory Animals Required and Justification:

1. Non-animal Alternatives Considered: No computerized model exists to simulate or reproduce the results that a study of a sentinel population of animals can produce. Cell culture models or computer models cannot be utilized to simulate the epidemiologic evaluation of a unique animal population for long term risks these animals may have encountered from their Service in the Persian Gulf.

2. Animal Model and Species Justification: The study does not require the purchase of any additional animals. It judiciously takes advantage of an extremely valuable animal model system already in place within the DoD. This animal model is the DoD MWD. These dogs are either German shepherd dogs or Belgian Malinois. The DoD MWD population has anatomic and physiologic systems similar to humans. MWD accompany many US Armed Forces Service members into various regions of the world under a wide spectrum of environmental conditions and operational scenarios. These sentinels afford an opportunity to look at physiologic, neurologic, clinical, and pathological factors that may be different from those observed in the nondeployed MWD. Furthermore, insight into possible environmental factors inherent to SWA and more specifically to the KSA may influence US troop deployment rotations, movement in theater, and future deployments and contingency operations.

3. Laboratory Animals:

a. Genus & Species: *Canis familiaris*

b. Strain/Stock: German shepherd dog and Belgian Malinois

c. Source/Vendor: NA

d. Age: 12 and 13 years

e. Weight: Varies, approximately 60-110 pounds

f. Sex: male and female

g. Special Considerations: Dogs are retired from active duty.

h. Other:

4. Total Number of Animals Required: 3

This protocol was designed to take full advantage of the opportunity to study the 118 veterans and 472 age, gender, and breed-matched nondeployed control dogs. At the onset of the original protocol, 88 PG deployed dogs had already died and been necropsied. Only 30 deployed MWD and their 120 nondeployed controls remained to be examined. Since then, most of the veteran and all of the control animals have also died and were examined under the original protocol. For this update, only three PG veterans remain to be studied.

5. Refinement, Reduction, Replacement:

a. Refinement: All MWD are humanely maintained. Procedures performed on these animals are routine diagnostics that could be performed on any MWD.

b. Reduction: The study includes only those PG veterans and their controls. No additional animals are required.

c. Replacement: There is no suitable replacement for this sentinel system.

C. Technical Methods:

1. Prolonged Restraint: NA

2. Surgery:

a. Procedure to include who will be performing the surgery: NA

b. Pre- and Post-operative Provisions to include who will be providing the care: NA

c. Location: NA

d. Multiple Survival Surgery Procedures: NA

(l) Procedures: NA

(2) Scientific Justification: NA

3. Animal Manipulations: Blood will be drawn for CBC, serum chemistry panel, serum acetylcholinesterase levels and thyroid function. Radiographs of multiple joints will be performed to complete the medical record. A neurologic examination

in accordance with the Colorado State University protocols (Tab A, Annex 1) and a behavioral assessment in accordance with the protocol in Tab A, Annex 2 will be performed. At necropsy, specimens of biceps femoris and triceps brachii muscle and tibial and radial nerve will be collected.

a. Injections: Animals will be anesthetized while radiographic procedures are performed. Animals will be premedicated and anesthetized according to the following or a similar protocol. Preanesthesia: Butorphanol 2.0 mg/ml, Acepromazine 0.5 mg/ml, and Glycopyrrolate 0.05 mg/ml: given at 1ml/20lbs. Induction: 100 mg ketamine and 10 mg diazepam. Maintenance: 2.0% isoflurane.

b. Biosamples: Tissue specimens for toxicologic analysis will be taken at necropsy (lung, liver, kidney, fat, and brain) on those dogs still living at the initiation of this protocol.

c. Animal Identification: All MWD are identified with a unique alphanumeric tattoo.

d. Behavioral Studies: A behavioral assessment will be performed on all living dogs included in this study in accordance with the protocol at Tab A, Annex 2.

e. Other Procedures: NA

4. Adjuvants: NA

5. Study Endpoint: Death of the animal at the end of its natural life is the endpoint. Death may be spontaneous or by euthanasia, the latter only for humane reasons as determined by the responsible veterinarian. Lifespan determination is an objective of this study.

6. Euthanasia:

a. Type: IV administration of Beuthanasia-D Special (Pentobarbital 390 mg/ml and Phenytoin 50 mg/ml.)

b. Who will perform the procedure: The attending veterinarian.

7. Pain:

a. USDA (Form 18-3) Pain category:

(1) No Pain 3 - 100%

(2) Alleviated Pain _____(#)_____%

(3) Unalleviated Pain or Distress _____(#)_____%

b. Pain Alleviation:

(1) Anesthesia/Analgesia/Tranquilization to include who will be administering it: As described in C.3.a Injections- above. Administered by the attending veterinarian.

(2) Paralytics: NA

c. Alternatives to Painful Procedures: NA

(1) Source(s) Searched: NA

(2) Date of Search: NA

(3) Key Words of Search: NA

(4) Results of Search: NA

d. Painful Procedure Justification: NA

D. Veterinary Care:

1. Husbandry Considerations:

a. Study Room: Animals will be housed in individual kennels at the DODMWDVS, Lackland, AB, TX.

b. Special Husbandry Provisions: None necessary.

2. Attending Veterinary Care: All animals are monitored daily by DODMWDVS personnel and evaluated by the center's veterinary staff.

3. Enrichment Strategy:

a. Dogs: Dogs are exercised three times a week and groomed three times a week by the center's handlers.

b. Nonhuman Primates: NA

VI. BIOHAZARD/SAFETY: NA

VII. ASSURANCES: As the Primary Investigator on this protocol I acknowledge my responsibilities and provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the IACUC.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous work.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used".

D. Biohazard\Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations. Documentation concerning completion of this training has been submitted to the AFIP Research Office.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Original Signed)

LTC Dale G. Dunn
(P.I. Signature)

G. Painful Procedures: NA

(Original Signed)

LTC Dale G. Dunn
(P.I. Signature)

Appendix C. Gulf War Study Protocol Data Abstraction Sheet

Gulf War Study Protocol

Tattoo: | **Name:** | **Breed:**

Sex: | **Date Neutered/Spayed:** | **Reason:**

Date of Birth: | **Date of Procurement:**

Departure Date from LAFB: | **Died/Euth:**

Date of Death: | **Clinical Cause of Death:**

Assignments:

Date		Location

Deployments: (OCONUS)

Date		APO/Unit/Location/Attending VS

Gulf war cohort Yes ☐ No ☐ **Returned to LAFB** Yes ☐ No ☐

**********If MWD died after Dec 1996, fill in the boxes below**********

Thyroid Panel:

Date	Free T4 SI	Free T4 ED	Reference Range	T4	Reference Range	CTSH	Reference Range	Lab Name

ACHase:

Date	Value

Peripheral Nerve:

Date	Affected	Unaffected

Clinical Neuro Exam Attached Yes ☐ No ☐ **Initials:** ☐

Appendix D. MWD Medical Records Microfilm Data

HEADER DATA

NO/NAME: _____/_____

BREED: LABRADOR GERMAN SHEPHERD MALINOIS BEAGLE MIX/OTHER_____

SEX: INTACT MALE NEUTERED MALE INTACT FEMALE SPAYED FEMALE

DATE OF BIRTH: (MM/DD/YY) _____/_____/_____

STATE OF ORIGIN: _____

DATE OF PROCUREMENT: (MM/DD/YY) _____/_____/_____

DATE OF DEATH: (MM/DD/YY) _____/_____/_____

CAUSE OF DEATH: (EUTHANIZED) _____ OR (DIED) _____ **PATH:** _____

KEY:

APPENDICULAR DJD	**ADJD**	ANESTHETIC ARREST	**ANES**
NEOPLASTIC	**NEO**	AXIAL DJD	**XDJD**
SPINAL CORD	**NEURO**	RESPIRATORY	**RESP**
GERIATIRIC (OLD AGE)	**GERI**	TRAUMA	**TRAU**
GDV	**GDV**	DERMATOLOGICAL	**DERM**
CARDIAC	**CARD**	BRAIN DISEASE	**BRN**
BEHAVIOR	**BEHV**	HEAT STROKE	**HEAT**
UROGENITAL	**URO**	ENDOCRINE	**ENDO**
GASTROHEPATIC (NON GDV)	**DIG**	MISCELLANEOUS	**MISC**
OPTHALMALOGIC	**OPTH**	UNDETERMINED/UNKNOWN	**UNK**

Appendix E. MWD Gulf War Study Checklist

NAME/TATTOO:_____

PROCEDURE	DATE	CLINICIAN
Arrival Physical Exam		
CBC/Chemistries		
Cholinesterase Activity		
EKG		
UA		
Fecal		
Neurologic Exam		
Cranial Nerves		
Proprioception		
Radiology		
Hips		
Stifles		
Elbows		
Spine		
Electrodiagnostics		
EMG		
NCV		
Spec EEG		
Behavior Exam		
Necropsy Performed		
Tissues Submitted		
Pathology Report Received		

Appendix F. Database Codebook

VARIABLE	Contents	Format
LTR	Fiscal Year Procurement Group	Text, 1 characters
CTATTOO	Tattoo Identification	Alphanumeric, 4 characters
CNAME	Canine Name	Text, 13 characters
BREED	Canine Breed	Text, 18 characters
BRD_CODE	Breed 0 - Belgian Shepherd Dog 1 - German Shepherd Dog 2 - Other Breeds	Numeric, 1 digit
CSEX	Canine Gender FS - Female, Spayed MI - Male, Intact MN - Male, Neutered	Text, 2 characters
SEX_CODE	Sex 0 - Female, Spay 1 - Male, Neutered 2 - Male, Intact	Numeric, 1 digit
YOB	Year of Birth	Numeric, yy, 2 digits
DDOB	Date of Birth	dd-month-yy, 9 characters
NDOD	Date of Death	dd-month-yy, 9 characters
YOD	Year of Death	Numeric, yy, 2 digits
AGE_YRS_	Age at Death (years)	Numeric, 4 digits
FAILURETIME	Total observation time (years)	Numeric, 4 digits
OCCUP	Occupation D - Drug Detector E - Explosive Detector P - Patrol PD - Patrol/Drug PE - Patrol/Explosive PES - Patrol/Explosive/Scout PS - Patrol/Scout S - Scout	Text, 3 characters
LGULFVET	Gulf Veteran	T/F, 1 digit
GVCODE	Veteran Code 0 - No 1 - Yes	Numeric, 1 digit
GWCOHORT	Gulf War cohort	T/F, 1 digit
WWL_CODE	Assignment Location 0 - CONUS 1 - SWA 2 - OCONUS	Numeric, 1 digit

WWL2	Regional Location 0 - CONUS 1 - SWA 2 - Europe 3 - Pacific 4 - Americas	Numeric, 1 digit
CLOCODE	SWA Assignment Location 0 - Dhahran AB, KSA 1 - Al Jubail AB, KSA 2 - Al Kharj AB, KSA 3 - Al Minhad AB, UAE 4 - Doha AB, Qatar 5 - Jeddah, KSA 6 - Khamis AB, KSA 7 - Kuwait 8 - Riyadh, KSA 9 - Shakisa, Bahrain	Numeric, 1 digit
LOC3	SWA Location 0 - Unexposed 1 - Kingdom of Saudi Arabia 2 - Other SWA Countries	Numeric, 1 digit
LENGTH	Time Spent in SWA (days)	Numeric, 3 digits
ARRIVAL	Period of Arrival to SWA 0 - No exposure 1 - Pre-War (1 Aug 1990 - 31 Dec 1990) 2 - During the War (1 Jan 1991 - 31 Mar 1991) 3 - Post - War (1 April - 31 Dec 1991)	Numeric, 1 digit
DEPART	Period of Departure to SWA 0 - No exposure 1 - Pre-War (1 Aug 1990 - 31 Dec 1990) 2 - During the War (1 Jan 1991 - 31 Mar 1991) 3 - Post - War (1 April - 31 Dec 1991)	Numeric, 1 digit
BRANCH	Branch of Service USA - US Army USAF - US Air Force USMC - US Marine Corps USN - US Navy	Text, 4 digits
SVC_CODE	Branch of Service Code 0 - USAF 1 - USA 2 - USN 3 - USMC	Numeric, 1 digit
ENTERAGE	Age at cohort Entry (years)	Numeric, 4 digits

MODE_OF_DEATH	Death Status AD - Adopted AL - Alive DH - Died in Hospital DS - Died Spontaneously EU - Euthanized	Text, 2 digits
MODECODE	Death Status Code 0 - Alive 1 - Died 2 - Euthanized	Numeric, 1 digit
CODE2	Diagnosis Code 1 - Alive or Lost to Follow-up 2 - Anesthetic 4 - Gastric Dilitation Vulvulous 5 - Cardiovascular 6 - Axial DJD 7 - Appendicular DJD 8 - Neoplasia 9 - Neurologic 10 - Respiratory 11 - Ophthalmologic 12 - Trauma 13 - Urogenital 14 - Dermatologic 15 - Digestive 16 - Geriatric 17 - Miscellaneous 18 - Endocrine 19 - Heat 20 - Brain 22 - Behavior 50 - Unknown	Numeric, 2 digits
DIAGNOSIS	Cause of Death/Euthanasia Category Abbreviations-- See Code2	Text, 5 digits
CODE3	Neurologic Mortality 0 - Censored 1 - Neurologic Mortality	Numeric, 1 digit
AFIPN	AFIP Neurologic Diagnosis 0 - unaffected 1 - affected	Numeric, 1 digit
BPNDX	Peripheral Nerve Disease Diagnosis	yes/no, 3 digits
BPN_CODE	Peripheral Nerve Disease Code 0 - No 1 - Yes	Numeric, 1 digit

THYROIDT4	Serum Total T4 Levels (μg/dl)	numeric, 3 digits
FREET4	Serum Free T4 Levels (ng/dl)	numeric, 4 digits
CTSH	Serum Canine TSH Levels (ng/ml)	numeric, 3 digits
CHOLINE	Serum Acetylcholinesterase Levels (IU/ml)	numeric, 3 digits
NXAM	Neurologic Exam Outcome 0 - unaffected 1 - affected	numeric, 1 digit
TIBVEL	Tibial Nerve Velocity (meters/second)	numeric, 2 digits
ULNVEL	Ulnar Nerve Velocity (meters/second)	numeric, 2 digits
HYPO	Hypothyroid Status 0 - normal 1 - hypothyroid	numeric, 1 digit
TIMEQ4	Length of Stay in Quartiles 0 - Unexposed (no time in SWA) 1 - 1st Quartile (30 to 92 days) 2 - 2nd Quartile (93 to 122 days) 3 - 3rd Quartile (124 to 176 days) 4 - 4th Quartile (177 to 304 days)	numeric, 1 digit