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Relationship between dysphoric moods, risk-taking behaviors, and Toxoplasma gondii antibody titers in female veterans

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Relationship Between Dysphoric Moods, Risk-Taking Behaviors, and *Toxoplasma gondii* Antibody Titers in Female Veterans

by

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DEDICATION

I would first like to dedicate this work to my committee. If it weren't for the four of you, I would not be in the position I am today. Your guidance and encouragement has made this process extremely educational and worthwhile. I have learned so much from all of you.

I would also like to dedicate this work to my husband. He has only known me as a student and has always supported me throughout this entire process.

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ABSTRACT

The number of female veterans is increasing daily. Previous research conducted on veterans has focused primarily on males or with small samples of females. Depression and suicidality are becoming increasingly evident in returning veterans. *Toxoplasma gondii* is an intracellular parasite that is common in the Middle East and has been reported to cause changes in personality and behaviors.

The purpose of the current study was to examine relationships between *T. gondii* antibody titer and socio-demographic variables, dysphoric moods, and risk-taking behaviors in a sample of 70 female veterans. Blood samples were collected and analyzed for *T. gondii* antibody titer and participants completed a battery of questionnaires, including the Center for Epidemiologic Studies Depression (CES-D) scale, Profile of Mood States (POMS), PTSD Checklist-Military version (PCL-M), Alcohol Use Disorder Identification Test (AUDIT), and a sexual harassment and assault questionnaire.

Results of chi-squares showed a relationship between *T. gondii* titer, ethnicity, marital status, and level of education. Pearson's correlations and t-tests showed relationships between *T. gondii* titer and POMS depression, confusion, and anger subscales, and total mood disturbance score.

CHAPTER ONE

INTRODUCTION

Toxoplasma gondii, an intracellular protozoan parasite, is the most common parasite found in developed nations, infecting approximately one-third of the world's population (Flegr, 2007). The seroprevalence rate of toxoplasmosis is estimated to be between 20%-80% worldwide, though latency is the most common state of infection (Dalimi & Abdoli, 2012). The Centers for Disease Control and Prevention (2013) classified *T. gondii* as the leading cause of death by foodborne illness in the United States and it is considered to be one of five neglected parasitic infections. More than 60 million individuals living in the United States have positive *T. gondii* antibody titers, which is an overall prevalence of 22.5%, ranging widely with geographic location and ethnicity (CDC, 2013).

T. gondii is only reproduced in the intestines of any member of the feline family. Therefore felines are the final hosts of the parasite. Any warm-blooded mammal, including humans, may be the intermediate hosts of the parasite. The life cycle of *T. gondii* includes three infectious stages: oocysts (the products of sexual reproduction of the parasite), tachyzoites (form of the parasite that is rapidly replicating and found in the acute phase of toxoplasmosis) and bradyzoites (form of the parasite slowly dividing and contained in tissue cysts, found during the latent phase of toxoplasmosis). The initial infection may be either asymptomatic or have flu-like symptomatology. After the initial, acute infection, the rapidly reproducing tachyzoites become the slowly dividing

bradyzoites, forming cysts that can be found in various tissues in the host's body, including skeletal muscle and brain tissue (Weiss & Kim, 2007).

The latent form of the disease has been associated with various personality and behavioral changes, both in rodents and humans. In rodents, behavioral changes include performing actions that make them preyed upon more easily. This manipulation of behaviors is hypothesized to occur in order for the parasite to perpetuate its own life cycle, supporting the "behavioral manipulation" hypothesis (Dalimi & Abdoli, 2012). These behaviors include delayed reaction time and loss of fear of predators (felines). Delayed reaction time and diminished psychomotor performance have also been reported in humans (Flegr, Novotna, Lindova, & Havlicek, 2008; Havlicek, Gasova, Smith, Zvara, & Flegr, 2001). Positive *T. gondii* antibody titer has been associated with psychiatric disorders, as well, such as schizophrenia, depression, and bipolar disorder (Flegr, 2013; Pearce, Kruszon-Moran, & Jones, 2012).

In this time of extreme military conflict, the number of United States military personnel is increasing. Psychosocial distress among this population is severe and it has been found that veterans are at a higher risk for developing, post-traumatic stress disorder (PTSD), depression, suicidal ideation (Mansfield, Bender, Hourani, & Larson, 2011), and engaging in risk-taking behaviors (Strom et al., 2012). As female veterans experience longer and more intense combat exposure than previously seen, the risk for developing psychosocial distress increases. In addition, as more female veterans are integrating back into civilian life, increased information is needed regarding the mental health needs of this population.

Women have a higher incidence of depression, suicidality, and military sexual trauma (MST) than their male counterparts (Nunnink et al., 2010; Haskell et al., 2010; McFarland, Kaplan, & Huguet, 2010). Suicide is a serious issue that is gaining attention as more and more veterans are returning home from deployments in areas of war. Female veterans, ages 18-34, were three times more likely to commit suicide than nonveterans (McFarland, Kaplan, & Huguet, 2010). In addition, military sexual trauma (MST) is another topic that requires additional research. Approximately 1 in 5 women and 1 in 500 men have reported some form of MST while actively serving in the military (Burgess, Slattery, & Herlihy, 2013).

While research has been conducted pertaining to veterans and psychological symptoms such as PTSD, depression, and deployment history, virtually no information is known about relationships of these factors and *T. gondii*, particularly in a sample of female veterans. The relationship between risk-taking behaviors and military service has been examined, however, the relationships between risk-taking behaviors and positive *T. gondii* IgG antibody titers in veterans has not. American soldiers may have an initial acute infection, often without symptoms, and then develop the chronic form of the disease, which is associated with personality and behavioral changes. By studying female veterans, it may be possible to determine not only the prevalence of infection in this population, but also if there is a relationship between *T. gondii* infection status and dysphoric moods and risk-taking behaviors.

Statement of the problem

The United States is currently engaged in military conflicts. Military personnel are being deployed and returning home after combat exposure every day. Trauma is frequently experienced by this population and increases the risk of dysphoric moods and psychosocial distress. As the number of female veterans increases, the need for more information regarding their mental health also increases. *T. gondii* infection is a relevant concern that requires more attention. Research and data for veterans and possible relationships between dysphoric moods, behavioral changes, and positive *T. gondii* antibody titers is extremely limited. Having a positive *T. gondii* antibody titer puts individuals at risk for psychiatric and neurological diseases, such as schizophrenia and bipolar disease, as well as increase risk of suicide. Identifying factors that have a relationship with positive *T. gondii* antibody titers will uncover information about the distress female veterans are experiencing. The purpose of this research was to investigate dysphoric moods, PTSD, risk-taking behaviors, and the relationships these factors may have with positive *T. gondii* IgG antibody titers in a sample of female veterans.

Specific aims with research questions

The proposed research question was "To what extent is there a relationship between positive *Toxoplasma gondii* antibody titers, demographics, dysphoric moods, PTSD and risky behaviors in female veterans?" The specific aims for this research were as follows:

Aim #1- To examine relationships of ethnicity, race, marital status, age, level of education and deployment history with *Toxoplasma gondii* antibody titer.

Aim #2- To examine relationships of *Toxoplasma gondii* antibody titer with dysphoric moods and PTSD.

Aim #3- To examine relationships of *Toxoplasma gondii* antibody titer with smoking, alcohol use, and sexual assault/ harassment.

Definition of relevant terms

The following terms have been defined for the purposes of this study:

Socio-demographic factors are defined as race, ethnicity, age, level of education, and marital status.

Ethnicity is defined as Hispanic and non Hispanic. *Race* is defined as Caucasian, African American, Asian/ Pacific Islander, and Eskimo/ Native American.

Dysphoric mood is defined as depressive symptoms, anger and hostility, as measured by the Profile of Moods States (POMS) questionnaire, Center for Epidemiologic Studies Depression (CES-D) Scale, and the PTSD Checklist Military Version (PCL-M).

Depression is defined as a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration (World Health Organization, 2012).

Post-traumatic stress disorder (PTSD) is defined as having a history of exposure to a traumatic event and meeting two criteria and symptoms from each of three symptom clusters: intrusive recollections, avoidant/numbing symptoms, and hyper-arousal symptoms (American Psychiatric Association, 2000).

Positive Toxoplasma gondii antibody titer is defined as a titer of 10 IU/mL or higher which indicates the presence of latent infection (Flegr, Lenochova, Hodny, & Vondrova, 2011).

Risk-Taking Behaviors- is operationalized by alcohol use, smoking status, and sexual harassment and assault.

Relevance and Significance

The healthcare system in the United States is experiencing an influx of military personnel requiring assistance, both in the Veterans Administration System and the civilian system. As the number of female veterans increases, the healthcare field must be prepared for this population as they are experiencing trauma and events differently than that of their male counterparts. With the increase in exposure of combat and trauma that may occur while serving in the military, potential sequelae such as depression and PTSD is more likely to occur. There is also an increase in engaging in risk-taking behaviors such as alcohol misuse and unsafe driving behaviors. *Toxoplasma gondii* is a parasite that infects a large portion worldwide. Deployment overseas increases the risk of *Toxoplasma gondii* infection in the military population and infection by this parasite has reportedly been associated with psychiatric disorders, such as schizophrenia and bipolar disease, as well as suicidality. In addition, it has been reported that both military personnel as well as individuals infected by *T. gondii* engage in behaviors that are considered risk-taking. The findings reported from this study provide data to assist in identifying factors that have a relationship with positive *T. gondii* antibody titers, including dysphoric mood and risk-taking behaviors in a sample of female veterans.

CHAPTER TWO

REVIEW OF LITERATURE

A comprehensive literature review using Academic Search Premier, Cochrane Database of Systemic Reviews and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials, MEDLINE, PsycINFO, and PubMed databases was conducted using the search terms of *Toxoplasma gondii*, female veterans, post-traumatic stress disorder, depression, deployment history, and risk-taking behaviors. This review of literature will outline prevalence and pathophysiology of *T. gondii*, *T. gondii* and psychological and behavioral changes, deployment history and *T. gondii* infection, military and psychological distress, military sexual trauma, and military and risk-taking behaviors.

***Toxoplasma gondii*- Prevalence and Pathophysiology**

Toxoplasma gondii, an obligate, intracellular parasite, is the most common parasite found in developed nations (Flegr, 2013). The prevalence of infection in the United States is approximately 22.5%, or 60 million individuals with a positive *T. gondii* antibody titer. The Centers for Disease Control and Prevention (2012) classified *T. gondii* as a neglected parasitic infection, in a group of five parasitic diseases that have been targeted by the CDC for public health action. Sexual reproduction of this parasite occurs only in the intestines of felines, and the feline host excretes the product of reproduction, the oocyst, in its feces (Dubey & Jones, 2008). The oocyst has been found to be virtually resistant in the environment for up to a year, with the potential for contaminating food

and water, and infecting other warm-blooded animals and humans (Carruthers & Suzuki, 2007; Kamerkar & Davis, 2012).

Undercooked meat has been considered a main source of infection, due to the fact that most farm animals that are seropositive for *T. gondii* have been shown to harbor infectious parasites in their muscles (Kijlstra & Jongert, 2008). Infection may also occur with the ingestion of unwashed fruits and vegetables that are found to contain oocysts, contaminated water or through handling litter boxes of infected cats (Ling, Lester, Mortensen, Langenberg, & Postolache, 2011). Eating raw oysters, clams, or mussels has been recently discovered to be a new risk factor as well (Jones et al., 2009).

During an initial acute infection by this intracellular parasite, tachyzoites rapidly replicate. This rapidly dividing and motile form of the parasite is contained in a number of the host's cells, including macrophages (Flegr, 2013). After the initial insult by tachyzoites, the second stage consists of slower growing bradyzoites, which form cysts . These cysts can be found primarily in brain tissue and skeletal muscle and remain latent for the remainder of the host's life (Kamerkar & Davis, 2012). The presence of living parasites protects the host against new infection, however, reactivation may occur when the immune system is compromised, such as in AIDS or immunosuppression in transplant or cancer patients (Kamerkar & Davis, 2012). Until recently, the chronic phase of the infection was thought to be completely asymptomatic. However, it is in this latent, chronic phase that mood and behavior changes have now been reported (Flegr, Kodym, & Tolarova, 2000; Flegr, 2007; & Arling et al., 2009).

T. gondii requires tryptophan, an essential amino acid for cell division and infectivity. One mechanism for accomplishing continuing latency is tryptophan stealing

from the infectious cysts so that their growth is thwarted and bradyzoites do not escape into the circulation. There is a continuous vigorous inflammatory cellular infiltrate and cytokine secretion around T. *gondii* cysts, in particular Interferon gamma (IFN-γ), Interleukin-12 (IL-12), and Tumor Necrosis factor-alpha (TNF-α), which activate the enzyme indoleamine 2,3-dioxygenase (IDO) (Pfefferkorn, 1984; Miller, Boulter, Rowan, Ikin, & Smith, 2009). Activation of IDO directs tryptophan into the kynurenic acid pathway and away from the production of serotonin. Another pathway inhibited through neurotropic inflammation is the guanosine triphosphate cyclohydrase -1 (GTP-CH1) enzyme which results in decreased tetrahydrobiopterin (BH4), a coenzyme to tryptophan and phenylalanine hydroxylases. There is additional inhibition of serotonin synthesis through this effect. A decrease in the concentration of serotonin could be responsible for depression and irritability in individuals infected by *T. gondii* (Flegr, 2013).

T. gondii and personality and behavioral changes

Personality and behavior changes have been examined in the *T. gondii* positive population. In its chronic, latent form, *T. gondii* has been shown to induce behavioral and personality changes in both rodents and humans. In rodents, observed changes in behavior include decrease in anxiety and neophobia, increase in activity and aggressiveness, and prolongation of reaction, all of which increase risk of predation (Vyas, Kim, Giacomini, Boothryd, & Sapolsky, 2007). *T. gondii* has been associated with the behavioral manipulation hypothesis. This hypothesis states that the parasite has the ability to manipulate rodent behavior in order to enhance transmission to its definitive host- the cat (Berenreiterova, Flegr, Kubena, & Nemec, 2011). Behaviors that are "manipulated" include prolongation of reaction time, loss of fear response to cat odor,

and increase in activity. Not only is there a loss of fear of a known predator, there is actually an attraction to the odor and sexual arousal pathways of the brain are activated (Flegr, Lenochova, Hodny, & Vondrova, 2011; House, Vyas, & Sapolsky, 2011).

In humans, *T. gondii* has been reported to be correlated with various personality and behavioral changes. Compared to uninfected men, men who were discovered to have a positive *T. gondii* antibody titer were more introverted, suspicious, and inclined to disregard rules (Flegr, 2007). Women, on the other hand, were found to exhibit opposite behaviors, including trusting, extroverted, and rule abiding when compared to non-infected women (Flegr, 2007; Lindova, Priplatova, & Flegr, 2012). In addition to these alterations in personality, psychomotor performance has also been examined and found altered in infected individuals, both male and female (Flegr, Havlicek, Kodym, Maly, & Smahel, 2002; Novotna et al., 2008). Flegr, Klose, Novotna, Berenreitterova, and Havlicek (2009) conducted a large-scale prospective study in which the authors examined military drivers for *T. gondii* seroprevalence. The authors reported an increased risk of traffic accidents in a sample of 3,890 military drivers in *T. gondii* infected drivers in Prague. Yereli, Balcioglu, and Ozbilgin (2006) also conducted a prospective study and reported that traffic accidents were also associated with positive *T. gondii* titer in in a sample of 185 participants in Turkey.

Suicide and suicide attempts and relationships with a positive *T. gondii* titer have been examined. It has been reported that suicide attempters had a significantly higher IgG antibody levels to *T. gondii* as compared with individuals without a suicide attempt (Arling et al., 2009). In a similar study conducted in Turkey, the seropositivity in level for anti *Toxoplasma* IgG antibodies of suicide attempt indicating chronic latent infection

was greater than in healthy volunteers (Yagmur, Yazar, Temel, & Cavusoglu, 2010). Ling, Lester, Mortensen, Langenberg, and Postolache (2011) obtained *T. gondii* seroprevalence and suicide rates from the European Mortality Database. The authors report that there is a positive relationship between rates of infection with *T. gondii* and suicide in women of postmenopausal age. Groer et al. (2011) examined 414 pregnant women for relationships between depression and positive *T. gondii* antibody titer. The authors reported that higher *T. gondii* IgG antibody titers in infected women were related to anxiety and depression during pregnancy.

T. gondii seroprevalence was also shown to have a significant relationship with bipolar disorder and schizophrenia (Holub et al., 2013; Pearce et al., 2012). Niebuhr et al. (2008) conducted a study to examine the relationship between schizophrenia and *T. gondii* seroprevalence in a sample 180 members of the U.S. military. In order to participate, the veterans had to have been hospitalized and discharged from military service with a diagnosis of schizophrenia. The authors reported a significant positive correlation between *T. gondii* IgG antibody titer and schizophrenia. Although the mechanism responsible for the change in personality and behavior is unclear, it has been hypothesized that they may occur as a result of increased levels of dopamine in the chronically infected brain. *T. gondii* contains 2 amino acids (hydroxylases) that have the potential to affect dopamine synthesis (Gaskell, Smith, Pinney, Westhead, & McConkey, 2009; Vyas, Kim, Giacomini, Boothroyd, & Sapolsky, 2007). In addition, it is hypothesized that metabolites are being released from the cysts found in the brain or from inflammatory processes that are occurring. The cysts may also

have a direct effect on the central nervous system, particularly the limbic system (da Silva & Langoni, 2009).

Deployment history and *T. gondii* infection

Deployment to the Middle East is associated with exposure to environment, food, and water that are likely to increase the possibility of *T. gondii* infection among soldiers. It has been reported that up to 50% of the meat sources of the Middle East such as camels and donkey have heavy *T. gondii* cyst contamination (Shaapan & Khalil, 2008) and the desert climate preserves the oocysts in cat feces. In addition, feral cats are ubiquitous in many areas of the Middle East. There may also be lower hygiene standards during deployment, contributing to the risk of infection by oocysts. American soldiers may have an initial primary infection, often without symptoms, and then develop the chronic form of the disease, which is associated with personality and behavioral changes. Although there is a definitive gap in the literature in regard to *T. gondii* infection and military personnel that have been deployed, a study was conducted with military personnel involved in jungle operations in Columbia (Gomez-Marin et al., 2012). Columbians in urban and jungle military personnel have a high prevalence of toxoplasmosis (24%) in a sample of 1001 soldiers. The authors hypothesized that this increased prevalence is likely due to consumption of contaminated water and undercooked meat.

Military and psychological distress

Psychosocial distress among veterans has been examined, and reported that military personnel are at a higher risk for developing post-traumatic stress disorder (PTSD) and depression, including suicidal ideation (Asmundson, Stein, & McCreary, 2002; Renshaw, Rodrigues, & Jones, 2009; Mansfield, Bender, Hourani, & Larson,

2011). The causes of depression, PTSD, dysphoric moods and suicide have been largely attributed to combat exposure and the horror and trauma of the battlefield, as well as military sexual trauma. In previous studies, approximately 12% to 20% of veterans returning from Iraq and Afghanistan screen positive for PTSD (Hoge et al., 2004). Overall, suicide rates among active-duty military serving as a part of Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) in Afghanistan have doubled since the beginning of the wars (U.S. Army, 2010). Deployment has been examined as a predictor of psychological distress in soldiers returning home from combat. Soldiers, who had been deployed to either Iraq or Afghanistan, have higher incidence of major depression, PTSD, or alcohol misuse after deployment than before (Hoge et al., 2004; Rudd, Goulding, & Bryan, 2011). In addition, anger, hostility, and aggression have been associated with soldiers returning home from deployments (Jakupak et al., 2007; Elbogen et al., 2010).

Women comprise approximately 14% of military personnel deployed in support of Operations Enduring Freedom and Iraqi Freedom (OEF/OIF), and women are increasingly exposed to military combat (Luxton, Skopp, & Maguen, 2010). However, most research has been conducted with male dominant samples. Few studies were found that compared male to female veterans. In an observational study conducted by Maguen, Luxton, Skopp, and Madden (2012), 7251 (6697 males and 554 females) active duty soldiers completed a demographic questionnaire, combat exposure questionnaire, and PTSD, depression, and alcohol use questionnaires at a large Army medical treatment facility. The authors reported that female soldiers reported an increase in depressive symptoms while male soldiers reported higher levels of alcohol misuse. Both depression

and PTSD have been cited as major problems among women in civilian populations, with women reporting higher incidence of depression and PTSD (Weissman et al., 1996; Kessler et al., 1995). In contrast, female veterans experience PTSD at similar rates to male veterans (Kang, Dalager, Mahan, & Ishii, 2005; Vogt et al., 2005). Hassija, Jakupcak, Maguen, and Shipherd (2012) conducted a cross-sectional study with 115 Gulf War and OEF/OIF female veterans presenting for healthcare services at a VA postdeployment specialty clinic. Post-traumatic stress disorder symptom severity, depressive symptom severity, and alcohol misuse were found to be higher among female veterans who reported combat exposure than female veterans who did not report combat exposure. Little is known about the association between combat exposure and psychological health outcomes for women who have been deployed to OIF and OEF. This limitation is extremely important, because for the first time, females are serving in combat alongside men in every capacity (Katz, Bloor, Cojucar, & Draper, 2007), thus increasing their exposure to trauma and risk for developing PTSD.

Military Sexual Trauma

Military sexual trauma (MST) is a serious health concern that affects a large portion of the U.S. military and causes distressing sequelae. The Department of Veteran Affairs (2013) defined military sexual trauma (MST) as repeated, threatening sexual harassment or sexual assault that occurred while in the military. The VA further describes MST to include "any activity of a sexual nature where someone is forced against his or her will, whether is occurred by pressuring the individual into sexual activities, being unable to consent to sexual activities, or being physically forced into sexual activities. Risk factors for MST include individuals who entered the military at a younger age, were

more likely to be of enlisted rank, and were less likely to have completed college (Sadler, Booth, Cook, & Doebbeling, 2003).

According to the Department of Defense (DOD), in the 2010 fiscal year, 3,198 service members reported that they were victims of a sexual assault. However, this number may be extremely underestimated. A cross-sectional study conducted by Grubaugh, Slagle, Long, Frueh, and Magruder, (2008) compared 183 African American female veterans and Caucasian female veterans for trauma exposure and psychiatric symptoms. Significant differences that were reported included the higher likelihood of Caucasian female veterans to endorse child sexual abuse compared to their African-American counterparts. The authors also report high rates of both adult sexual assault (49.3%) and child sexual assault (54%) in the entire sample of female veterans, both Caucasian and African American. There is an increasing amount of evidence that women who are exposed to sexual assault throughout their lifetime have poorer physical health, and/or poorer health perceptions, as well as greater psychologic difficulties, compared with those without traumatic experiences (Sadler, Booth, Neilson, & Doebbeling, 2000; Baker, Norris, Jones, & Murphy, 2009). Booth, Mengeling, Torner, and Sadler (2011) conducted a retrospective study in which 1,004 female veterans completed telephone questionnaires regarding substance abuse, rape history, depression and PTSD. The authors reported that the prevalence of lifetime rape in this population was 62%, in-military rape was 25%, and 34% reported a history of substance use that fit the criteria for DSM-IV criteria for abuse of dependence.

Military and Risk-taking Behaviors

Propensity for engaging in risk taking behaviors, including excessive alcohol consumption has also been reported as being increased in military personnel (Griffith & West, 2010; Hawkins, Lapham, Kinlahan, & Bradley, 2010). An exploratory study conducted by Strom et al. (2012) set out to determine if there is a relationship between symptoms of PTSD and risk-taking behaviors in 395 veterans. The authors reported that PTSD symptoms were significantly associated with elevated rates of risk-taking behaviors, alcohol use, and firearm possession. The study expanded the definition of risky behaviors to risky and aggressive driving practices, suicidal ideation, and sexual risk taking, as well as alcohol consumption, likelihood of physical violence, and possession of firearms. Kelley et al. (2012) examined 262 Army servicemen for risk propensity and health risk behaviors. The authors reported that participants with PTSD scored higher on aggression, risk-thrill seeking, and reported more drinks consumed during a drinking episode, more frequent drinking episodes, and using more alcohol than intended.

Combat exposure has been reported as a possible predictor of risk-taking propensity (Killgore et al., 2008; Kelley et al., 2012). Soldiers with higher levels of exposure to violent combat, who had killed another person, and had been exposed to intense human trauma were more willing to engage in risk-taking behaviors such as driving fast, taking dangerous shortcuts, alcohol use, and exhibiting physical and verbal aggression towards others (Killgore et al., 2008). Adler et al. (2011) conducted a study of 1,651 soldiers to assess the effect of the transition home from combat on risk-taking and health-related behaviors. The authors reported that participants were three times more

likely to engage in risk-taking behaviors, such as getting into a fight, carrying an unnecessary weapon, and engaging in unhealthy behaviors, such as drinking alcohol and smoking cigarettes. Soldiers returning from deployment have an altered perception of their ability to survive, their invincibility, which is understandable as they survived a uniquely dangerous period of life, subsequently diminishing their perception of susceptibility to negative consequences of risk-taking behaviors (Kelley et al., 2012).

Gaps in the literature

The preceding review of literature details the personality and behavioral changes that have been associated with *T. gondii* infection. The association between *T. gondii* infection and psychiatric disorders, such as schizophrenia and bipolar disease, should cause researchers to further investigate this relationship in a variety of populations. However, there is a gap in the literature in regards to veterans, both males and females, and *T. gondii* infection. Female veterans are increasing in number, actively serving, in the reserves, or retired. As with male veterans, this population has sequelae much different from the civilian population that requires more attention.

Summary

Military personnel have been extensively examined for physical and psychological consequences of service and deployment. It has been repeatedly reported that this population has an increased incidence of PTSD, depression, and suicidality. More specifically, the abundance of literature has been conducted with male veterans as the population of interest. However, there is an increased need for to address the gap when it pertains to female veterans. As the population of female veterans increases, their mental and physical health needs must be examined. Women represent a rapidly growing

and high PTSD-risk segment of the U.S. veteran population (Nunnink et al., 2010). Research on whether there is a relationship between these psychosocial measures such as depressive symptoms and PTSD, as well as risk-taking behaviors in female veterans with a positive *T. gondii* antibody titer has not been done. Given the larger percentage of women serving in Iraq and Afghanistan conflicts as compared to past wars, it is expected that increasing numbers of women will be subjected to the hardships of a war zone deployment, including exposure to combat and possible exposure to the parasite *Toxoplasma gondii*. A major limitation that has been cited in studies that include female veterans is that of small sample size. Small sample sizes limit the generalizability of the results to a national sample. Therefore, future research must attempt to include a larger and more diverse sample.

CHAPTER THREE

METHOD

The purpose of this research was to explore the relationships among dysphoric moods and risk-taking behaviors and positive *Toxoplasma gondii* antibody titer in a sample of female armed services veterans. Identifying potential psychosocial stressors experienced by female veterans and relationships among these psychosocial stressors and positive *T. gondii* antibody titer may provide information to improve mental health of female veterans. Previous research has suggested that there is a relationship between *T. gondii* infection and risk-taking behaviors as well as a number of psychiatric disorders such as depression and suicidality. Risk-taking behaviors are operationalized by self-report of current tobacco use, alcohol use, and sexual assault or harassment history. In regards to sexual assault and harassment operationalizing risk-taking behaviors, the circumstances to which these experiences occurred are unknown. However, it is possible that the participants in this study may have been in situations that increase the risk of sexual assault or harassment.

Chapter three describes the approaches used within the research. The chapter begins with the identification of the design with rationale and a description of the sample and setting. Instrumentation is described and validity and evidence of reliability of the scores are provided. Study procedures are described in detail to include Institutional Review Board approvals, recruitment, informed consent, and data collection procedures. Finally, the data analysis plan and rationale for the plan are presented.

Design

The study was a secondary data analysis of the primary cross-sectional, exploratory study, conducted to investigate relationships among behaviors, stressors, moods, and immune function in female veterans. The purpose of the proposed study was to explore potential relationships among dysphoric moods, risk-taking behaviors, and positive *T. gondii* antibody titers in female veterans. To date, no studies have examined relationships between *T. gondii* antibody titers and dysphoric mood, PTSD, and risk-taking behaviors in veterans. Therefore, the conceptual model was developed based on an extensive literature review. See Figure 1 for the conceptual model proposed in this study. According to the conceptual model, socio-demographic variables, such as age, marital status, ethnicity, race, and level of education, as well as history of at least 1 deployment were hypothesized to be related to *T. gondii* antibody titer. *T. gondii* antibody titer was also hypothesized to be related to dysphoric moods, including depressive symptomatology, anxiety, anger, and tension, post-traumatic stress symptomatology, and risky behaviors.

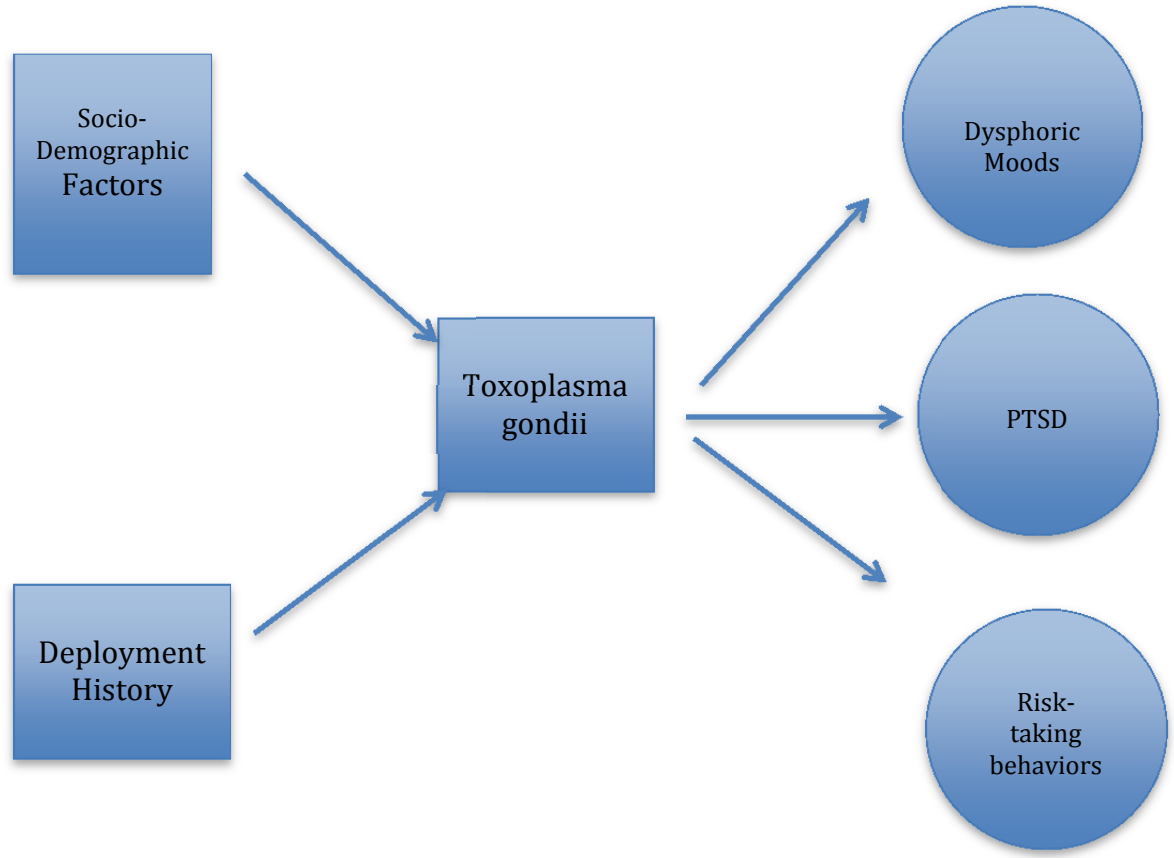


Figure 1. Conceptual Framework

Sample

The initial sample of female veterans (n=52) was given information about the research and provided informed consent upon arrival at a day of recognition for female veterans (event), given the opportunity to review the information, and were enrolled to participate at the event. The remainder of the sample (n=23) were given information about the research either by phone or email prior to setting the appointment and provided informed consent at the time of the appointment. Internet questionnaires were completed by all participants in the study after informed consent was provided. Participants had to be primarily English speaking, with the ability to read at an 8th grade level. As

participants were required to have served in the military, they have obtained at least a high school diploma or GED equivalent. Participating women received a $50 gift card.

Setting

The data were collected one time at the event at the Museum of Science and Industry in Tampa, Florida, as well as from December 2012 through May 2013 via individual appointments. Participants recruited from December 2012 through May 2013 either made individual appointments at the biobehavioral lab at the University of South Florida College of Nursing or were visited at their homes. This was determined by availability of transportation. Blood was drawn via venipuncture and hair was collected either at the event or at the individual appointments. Participants completed Internet questionnaires on touch screen computers at the event or after their individual appointments.

Instruments

Socio-Demographic questionnaire

All participants completed a demographic questionnaire. For the purposes of this analysis, ethnicity, race, age, marital status, and level of education were examined.

Deployment History

Deployment history was collected by the question "Have you ever been deployed?" and used as a dichotomous "yes" or "no" response.

Profile of Mood States

The Profile of Mood States (POMS) (McNair et al, 1992) is a 65-item instrument designed to elicit reporting of feelings over the past week, including the day of measurement. There are six subscales: tension-anxiety, depression-dejection, anger-

hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Experience of mood is reported on a 5-point summated rating scale, with responses of 0 (not at all), 1 (a little), 2 (moderate), 3 (quite a bit), and 4 (extremely). The POMS has been found to yield highly reliable and valid data in a number of studies of adults in a variety of life circumstances. The internal consistency ranges from .87 to .92 and test-retest reliably from .68 to .74 (McNair et. al., 1992). Research on the structure of mood (Diener & Emmons, 1984; Watson & Tellegen, 1985) consistently reveals two factors, positive affect and negative affect. The POMS was deemed ideal for assessing the whole range of moods in female veterans.

Posttraumatic Stress Disorder Checklist, military version (PCL-M)

The PCL-M is a 17-item, summated rating scale. The PCL-M was chosen to assess post-traumatic stress disorder in this sample due to its specificity to the military population and asks questions pertaining to symptoms in response to "stressful military experiences" (Weathers et al., 1993). Participants are asked to rate responses to stressful experiences as 1 (not at all), 2 (a little bit), 3 (moderately), 4 (quite a bit), 5 (extremely) and the items are summed for a total score. Total scores range from 17-85, with higher scores indicating higher levels of post-traumatic stress symptomatology. A score of 17-33 is indicative of low PTSD, 34-43 is indicative of moderate PTSD, and 44-85 indicative of high PTSD. The PCL–M has been shown to have excellent concurrent validity (r=.93) (Blanchard, Jones-Alexander, Buckley, & Forneris, 1996) and evidence of test–retest reliability (r=.96) (Weathers et al., 1993).

Center for Epidemiologic Studies Depression Scale (CES-D)

The CES-D is a 20-item, summated rating, widely used, self- report questionnaire that assesses depressive symptoms. Participants are asked to rate depressive symptoms over the last week as 0 (rarely or none of the time), 1 (some or a little of the time), 2 (occasionally or a moderate amount of the time), 3 (most or all of the time) and the items are summed for a total score. Total scores range from 0-60, with higher scores indicating higher levels of symptomatology. A cut-off score above 16, though not diagnostic, has been highly correlated with clinical depression (Radloff, 1977). The correlations between the CES-D items and total score have been reported to range from .40-.70 (Van Dam & Earleywine, 2011; Orme, Reis, & Herz, 1986). Evidence of reliability of the CES-D was provided by high total scale alphas for women in middle age life (α = .88) (Knight, Williams, McGee, & Olaman, 1997) and females veterans returning from Iraq and Afghanistan (α = .96) (Owens, Herrera, & Whitesell, 2009). Internal consistency was also assessed in a sample of soldiers returning from combat operations in Iraq and Afghanistan both Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) with high alphas (α = .92 for OIF and α =.93 for OEF) (Lapierre, Schwegler, & LaBauve, 2007). Test retest reliability was also tested after four weeks and was found to be r = .67 (Radloff, 1977).

Alcohol Use Disorder Identification Tool (AUDIT)

The AUDIT is a 10-item, summated rating, self-report questionnaire that assesses hazardous drinking and/or alcohol abuse. Participants are asked questions regarding frequency and number of alcoholic beverages consumed on a rating scale of 0-4, with a range of 0-40. Responses per question consisted of 0 (never or no), 1 (monthly or less or

less than monthly), 2 (monthly or 2-4 times a month), 3 (weekly or 2-3 times a week), or 4 (daily or almost daily). A score of 8 or more is associated with harmful or hazardous drinking. A score of 13 or more in women and 15 or more in men, is likely to indicate alcohol dependence (Babor, Biddle-Higgins, Saunders, & Monteiro, 2001; Saunders, Aasland, Babor, de la Fuente, & Grant, 1993).

Sexual Harassment and Assault

While developing the study, the research team searched for an instrument that would assess sexual harassment and assault history, both prior to the military and while in the military. No such instrument was found, and therefore, the team created an instrument to obtain that information. There were 4 questions asked, which included self-reports of episodes of sexual harassment in life both in and out of the military and episodes of sexual assault (rape or attempted rape) both in and out of the military. All 4 questions evaluated both occurrence and frequency of the harassment or assault. See Appendix F for Sexual Harassment and Assault questionnaire.

Smoking Status

Smoking status was collected by asking the participants if they were never smoked, if they were a previous smoker, or if they currently smoked.

Toxoplasma gondii

Participant's blood samples were collected at the female veteran's event or, if recruited later, at individual appointments. Blood was collected in three 5 milliliter heparinizes tubes and transported to the biobehavioral lab at the University of South Florida College of Nursing in a chilled cooler to be processed. Blood samples were spun

in a centrifuge at 1200 rpms for 25 minutes at 4˚C. In this process, plasma was separated, removed by pipette, and placed in Eppendorf tubes for storage. Plasma was stored at -80°C until the *T. gondii* assay was completed. Participant's blood samples were assessed for quantitative IgG antibodies. Titers were measured with a commercially available ELISA (Abcam Inc, Cambridge, MA). To obtain a 1:100 dilution, 10µl sample and 1 ml IgG sample diluent were dispensed into tubes and thoroughly mixed with a Vortex. Four standards and diluted samples (100µl) were dispensed in their respective wells, with well A1 left for substrate blank. Wells were covered with foil and incubated for 1 hour at 37 °C. After incubation was complete, contents of each well was aspirated, each well was washed 3 times with 300µl of washing solution, and 100µl of *Toxoplasma gondii* anti-IgG Conjugate was dispensed into all wells except the blank well. The plate was covered with foil and incubated for 30 minutes at room temperature. The wash step was repeated, 100µl of TMB Substrate Solution was dispensed into all wells and incubated for exactly 15 minutes at room temperature. Stop Solution (100µl) was dispensed into each well in the same order and at the same rate as the Substrate Solution and then measured at 450/620 nm. After further analysis of the IgG antibody titers, it was agreed that a second kit from a different manufacturer should be used to analyze the samples. The reason for this decision was that the standards tested in the original kit were unreliable at the cut-off titer of 10 IUs which was utilized as the indicator of infection. Similar procedures were used for the second ELISA as for the first one.

IgM antibody was assessed for participant's whose IgG antibody titer was above 10 International Units (IUs). Titers were measured with a commercially available kit (Abcam Inc, Cambridge, MA), according to kit directions.

Procedures

Dr. Maureen W. Groer, the principal investigator of the primary study gave permission for the researcher to utilize existing data.

Institutional Review Board (IRB)

Institutional Review Board approval was required for the secondary data analyses of the parent research. The IRB application was requested and approved as expedited under category 5 with a HIPPA waiver.

Contact/Recruitment

Initially, participants were recruited in the Tampa Bay area to attend the event through newspaper advertisements. Additional recruitment was accomplished through emails sent to veteran students at the University of South Florida Tampa campus. The Office of Veterans Affairs has access to approximately 1,500 students on campus and agreed to send information regarding the study to those students. The email sent was approved by the IRB. Communication among the study participants and their peers and colleagues was also a mode of recruitment.

Informed Consent

Informed consent was obtained at the one-day event or at individual appointments. Participants were given adequate time to fully and completely read the consent forms and ask questions if needed. Participants were provided a copy of the informed consent.

Data Collection

Data collection began at the female veterans' event at the Museum of Science and Industry November 2011 and resumed from December 2012 to May 2013 through

individual appointment. Questionnaires were completed via computer survey, using Checkbox 4.7 (Checkbox Survey Solution, Inc.) Blood was collected via venipuncture.

Data Analysis

Data analysis was performed to identify the relationship between demographic factors, POMS scores, PCL-M scores, CES-D scores, AUDIT scores, history of sexual assault and harassment, and smoking behaviors and *T. gondii* infection in female veterans. Descriptive statistics will be utilized to describe all of the study variables. As part of the secondary analysis, data will be cleaned in order to adjust for missing data. Data was examined for normality and transformed if necessary. Reliability data (α) for the instruments employed in this study were calculated and reported in the final analysis.

Aim #1- To examine relationships of ethnicity, race, marital status, age, level of education deployment history with *Toxoplasma gondii* antibody titer.

In order to assess the frequency of positive *T. gondii* antibody titers among these variables, Pearson chi-square tests were employed. A chi-square test examines the relationship between two categorical variables, and tested the observed frequency or proportion of cases that occur in *T. gondii* positive compared to *T. gondii* negative participants. The Pearson chi-square test was examined for asymptotic 2-sided significance, with $p \leq .05$ being considered significant. Pearson's product moment correlation coefficient (r) was employed to examine the relationship between *T. gondii* antibody titer and age of the participants. A *t*-test was also utilized to compare the *T. gondii* positively infected group and the *T. gondii* negatively infected group. *T. gondii* antibody titer was distinguished as positive or negative based on a titer of 10 or higher International

Units (IUs) (Jones, Kruszon- Moran, & Wilson, 2007).

Aim #2- To examine relationships of *Toxoplasma gondii* antibody titer with dysphoric moods and PTSD. Pearson's product moment correlation coefficient (*r*) was calculated to examine the relationship between the variables of interest and positive *T. gondii* antibody titer. In order to assess the difference between participants with a negative *T. gondii* antibody titer versus participants with a positive *T. gondii* antibody titer, a cutoff of 10 International Units (IUs) was used, splitting the total sample into two groups. *T*- tests were then used to compare the difference between the two groups and scores on the Profile of Mood States (POMS), Center for Epidemiologic Studies Depression (CES-D) scale, and the PTSD Checklist- military version (PCL-M). Effect size is the magnitude or a measure of the strength of the relationship between variables (Tabachnick & Fidell, 2007) and calculated for all *t*-tests conducted in this study.

Aim #3- To examine relationships of *Toxoplasma gondii* antibody titer with smoking, alcohol use, and sexual assault/ harassment.

Pearson's product moment correlation (*r*) was calculated to estimate the relationship between alcohol use and positive *T. gondii* antibody titer.

In order to assess the difference between participants with a negative *T. gondii* antibody titer versus participants with a positive *T. gondii* antibody titer, a cutoff of 10 International Units (IUs) was employed, splitting the total sample into two groups. *T*- tests were then used to compare the difference between the two groups in regards to alcohol use. Relationships between smoking status and sexual harassment/ assault and *T. gondii* antibody titer were examined by the Pearson

chi-square test for asymptotic 2-sided significance, with $p \leq .05$ being considered significant, as both smoking status and history of sexual harassment/ assault were analyzed as dichotomous variables.

CHAPTER FOUR

RESULTS

Preliminary Analyses

Missing data and normality. Blood samples were collected from 73 participants. Three out of 73 cases were excluded from analyses due to missing most if not all of the psychosocial data on the key variables. The final sample size for this study was 70 participants. Skewness was assessed in the *Toxoplasma gondii* antibody titer. *Toxoplasma gondii* IgG antibody titer positively skewed. Skewness is described as the asymmetry of the distribution and a variable is considered skewed when the mean is not in the center of the distribution. With positive skewness, there is a pileup of cases found to the left of the distribution and the right tail is too long. Logarithm (10) transformation was utilized to correct the skewness and obtain a normal distribution.

Participant age and total scores on the Center for Epidemiologic Studies Depression scale (CES-D), post-traumatic stress disorder checklist (PCL-M), and Profile of Mood States (POMS) were also assessed for normal distributions. PCL-M and POMS were both positively skewed and Logarithm (10) transformed.

Initial analysis of the data revealed that there were missing data on various items on the Profile of Mood States (POMS). As stated in Chapter 3, the POMS is a 65 item instrument with 6 subscales. In the cases with missing data, generally one item per subscale was missing. Mean substitution was chosen to estimate the missing values, as the mean is the best guess when in need of the value of a variable (Tabachnick & Fidell,

2007). A total of twenty-six questions or individual items were imputed for the entire sample.

Description of the sample. Participants ranged in age from 24 to 71 years (*M*=47.13; *SD*=10.60). Over half of the sample self-reported as Caucasian (n=42; 60.0%), while 18 participants (25.7%) self-reported as African American, 3 self-reported as mixed (4.3%), 1 as Asian/ Pacific Islander (1.4%), 1 as Eskimo/ Native American Indian (1.4%), and 5 as other (7.1%). See Table 1 for racial distribution of the sample. The ethnicity of the sample was primarily Non-Hispanic (n=59; 84.3%).

Table 1.

Race	N	%
Caucasian	42	60.0%
African American	18	25.7%
Asian/ Pacific Islander	1	1.4%
Eskimo/ Native American	1	1.4%
Mixed	3	4.3%
Other	5	7.1%

Additional demographic information analyzed in this study includes marital status, level of education, and deployment history. Twenty-seven participants (38.6%) reported that they were married, while 24 (34.3%) reported their marital status as divorced. Twelve participants (17.1%) reported that they were single or never married. Five participants (7.1%) reported that they were widowed and 2 participants (2.9%) reported that they were separated.

Eighteen participants (25.7%) reported completion of a Masters degree, 17 participants (24.3%) reported completion of a college degree, and 14 (20.0%) participants reported completion of a community college degree, while 12 (17.1%) participants reported completion of some college. Five (7.1%) participants reported completion of a doctoral degree, 3 (4.3%) participants reported completion of a high school diploma, and 1 (1.4%) participant reported a vocational or technical degree. Thirty-four participants (48.6%) reported that they had previously been deployed.

Psychosocial and Psychological variables. The mean score of the Profile of Mood States (POMS) total was 26.91 (*SD*=41.69) with scores ranging from -29 to 142. Table 2 summarizes the subscales of the POMS instrument. Scores on the Center for Epidemiologic Studies Depression Scale (CES-D) ranged from 0-45 (*M*=18.10; *SD*=47.06). A score above 16 indicates depressive symptomatology. Thirty-five participants (50%) had CES-D scores of 16 or higher. Participants were sent letters with results and explanations of abnormal biologic and psychologic variables and a list of resources to use.

The mean score on the PTSD Checklist- Military version (PCL-M) was 35.87 (*SD*=18.41) with scores ranging from 17-77. Scores ranging from 17-33 are indicative of low PTSD symptomatology, scores ranging from 34-43 are indicative of moderate PTSD symptomatology, and scores ranging from 44-85 are indicative of high PTSD symptomatology. Forty-two participants (60%) had scores of low symptomatology, 5 (7.1%) participants had scores of moderate symptomatology, and 23 (32.9%) participants had scores of high symptomatology. The mean score of the AUDIT instrument was 2.36

(*SD*=3.28). A score of 8 or above is indicative of hazardous or harmful alcohol intake. Six participants (9%) scored 8 or above.

History of sexual assault and harassment was collected from 4 questions. Forty-eight participants (68.6%) reported sexual harassment (e.g., made to feel inferior, ridiculed for their gender, denied job opportunities) in life outside the military. Fifty participants (71.4%) reported harassment in life in the military. Twenty-six participants (37%) reported sexual assault (e.g., rape, attempted rape) in life outside the military and twenty-three participants (32.9%) reported sexual assault while in the military. Fifty-eight (83%) participants reported that they never smoked or previously smoked, while 12 (17%) participants reported that they were currently smokers.

Toxoplasma gondii IgG antibody titer in this sample was analyzed on all 70 participants. The mean of the *T. gondii* titer was 18.03 IU (*SD*=47.06) with a range of 1.66-227.01. IgM antibody was assessed for participant's whose IgG antibody titer was above 10 International Units (IUs). Participants did not have new (primary) infection as verified by negative IgM titers. Untransformed raw data were used for the results described above. However, based on the skewness of the data, *T. gondii* IgG antibody titer was Log10 transformed. The cut point defining positive titer (and therefore infection) of 10 IUs was used for this analysis. Eight participants (11.42%) had an antibody titer higher than 10 IUs. Four Caucasian participants, 1 African American participant, 1 participant that reported mixed ethnicity, and 2 participants that reported "other" as their ethnicity had antibody titers higher than 10 IUs.

Table 2

Profile of Mood States summary of subscales

	Min Score	Max Score	*M*(*SD*)
POMS-Tension	0	30	8.79 (8.22)
POMS-Depression	0	43	9.60 (10.89)
POMS-Anger	0	40	6.69 (9.33)
POMS-Fatigue	0	28	9.46 (7.64)
POMS-Confusion	0	23	7.36 (4.75)
POMS-Vigor	0	30	14.97 (8.71)
POMS-Total	-29	142	26.91 (41.69)

Analyses Addressing the Study Aims

Aim #1. To examine relationships of ethnicity, race, marital status, age, level of education and deployment history with *Toxoplasma gondii* positive and negative infection status. Chi-square tests and a *t*-test were conducted in order to examine the existence of relationships between a positive or negative *T. gondii* antibody titer and the other variables of interest. The relationship between *T. gondii* and ethnicity was significant, X^2 (1, $N = 70$) = 11.90, $p \leq .05$. See Table 3 for the crosstabulation of this relationship. The relationship between *T. gondii* and education level was significant, X^2 (4, $N = 46$) = 9.24, $p \leq .05$. See Table 4 for the crosstabulation of this relationship. After calculation of the crosstabulation tables were completed, cells in the level of education table had frequencies of 0. In order to calculate an accurate chi-square, cells in which frequencies of 0 occurred were removed. Participants with a positive *T. gondii* antibody titer were significantly older than those with a negative *T. gondii* antibody titer $t(17) = 2.25$, $p \leq .05$, with an effect size (η^2) of .23. No significant results were found between *T. gondii* and deployment history, race, and marital status, therefore, they were not reported.

Table 3

Crosstabulation of T. gondii infection status and ethnicity

	Ethnicity			
T.gondii Status	Hispanic	Non Hispanic	X^2	ϕ
Negative	7 (.64)	55 (.93)	11.90**	.34
Positive	4 (.36)	4 (.07)		

NOTE: **-$p \leq .05$

Table 4

Crosstabulation of T. gondii infection status and level of education

T. gondii Status	Level of Education						
	High School	Some College	Community College	College Degree	Masters Degree	X^2	ϕ
Negative	2 (.67)	9 (.75)	13 (.93)	16 (.94)	17 (.94)	9.24*	.44
Positive	1 (.33)	3 (.25)	1 (.07)	1 (.06)	1 (.06)		

NOTE: **-$p \leq .05$

Aim #2. To examine relationships of *Toxoplasma gondii* IgG antibody titer with dysphoric moods and PTSD. Pearson's correlation (*r*) was calculated on the total range of *T. gondii* antibody titers, CES-D scores, PCL-M scores, and POMS scores, both total score and subscales. Total range of *T. gondii* antibody titers was significantly correlated with POMS depression subscale $r = .245$, n = 70, $p \leq .05$, POMS confusion/ bewilderment subscale $r = .242$, n = 70 $p \leq .05$, and POMS total subscale $r = .292$, n = 70 $p \leq .05$. See Table 5 for Pearson's correlations among all key variables. Scatterplots depicting the significant correlations can be found in Figures 2-5.

Also, t-tests were conducted on these variables, splitting the *T. gondii* titer at 10 IUs to create the 2 groups. Participants with a positive *T. gondii* antibody titer had a

significantly higher POMS depression score $t(68) = 2.35$, $p \leq .05$ with an effect size (η^2) of .08. Participants with a positive *T. gondii* antibody titer had a significantly higher POMS tension score $t(68) = 2.06$, $p \leq .05$ with an effect size (η^2) of .06. Participants with a positive *T. gondii* antibody titer had a significantly higher POMS anger score $t(68) = 2.18$, $p \leq .05$ with an effect size (η^2) of .07. Cronbach's alpha (**α)** was calculated for each instrument analyzed in this study and presented in Table 6.

Table 5

Correlations between Toxoplasma gondii antibody titer and psychosocial variables

	1	2	3	4	5	6	7	8	9	10
1 Toxotiter(IUs)	1.00	.197	.175	.245*	.105	.242*	.198	.197	.292*	.224*
2 PCL-M		1.00	.733**	.717**	.612**	.732**	.566**	-507**	.748**	.774**
3 POMS-Tension			1.00	.800**	.615**	.760**	.719**	-.617**	.828**	.691**
4 POMS-Depression				1.00	.679**	.736**	.704**	-.638**	.868**	.739**
5 POMS-Fatigue					1.00	.760**	.477**	-.671**	.781	.589**
6 POMS-Confusion						1.00	.573**	-.639**	.776**	.665**
7 POMS-Anger							1.00	-.390**	.708**	.536**
8 POMS-Vigor								1.00	-.801**	-.669**
9 POMS-Total									1.00	.770**
10 CES-D										1.00

*-$p \leq .05$ **-$p \leq .001$

Table 6

Cronbach's alphas for POMS, PCL-M, CES-D, and AUDIT

Instrument	Number of items	Cronbach's alpha (α)
POMS-depression	15	.94
POMS-tension	9	.93
POMS-anger	12	.94
POMS-confusion	7	.74
POMS-fatigue	7	.94
POMS-total	65	.91
PCL-M	17	.96
CES-D	20	.90
AUDIT	10	.82

Aim #3. To examine relationships of *Toxoplasma gondii* IgG antibody titer with smoking, alcohol use, and sexual assault/ harassment. The relationship between *T. gondii* antibody titer and smoking frequency was significant, X^2 (2, N =70) = 8.53, $p \leq .05$. See Table 7 for the crosstabulation of this relationship. A t-test was conducted to analyze *T. gondii* antibody titer and scores on the AUDIT. Participants with a positive *T. gondii* antibody titer had a significantly lower AUDIT score $t(68) = 3.20$, $p \leq .05$ with an effect size (η^2) of .24. t-tests were conducted on the *T. gondii* antibody titer and each item on the Sexual Assault and Harassment questionnaire. Chi-square tests were also conducted on each item on the Sexual Assault and Harassment questionnaire and *T. gondii* as a dichotomous variable (positive infection or negative infection). No significant results were found between *T. gondii* antibody titer and sexual assault/ harassment. Non-

significant t-tests are as follows: item 1 (experience sexual harassment in life outside of the military) $t(68) = .265$, $p = .792$; item 2 (experience sexual harassment in life in the military) $t(68) = .007$, $p = .994$; item 3 (experience sexual assault in life outside of the military) $t(68) = .006$, $p = .995$; item 4 (experience sexual assault in life in the military) $t(68) = -.193$, $p = .847$. All effect sizes for these relationships were less than .001.

Given that the responses to the sexual harassment and sexual assault were so high, McNemar tests were conducted to examine the differences in the participants who reported sexual harassment and sexual assault both in and out of the military. The McNemar test was not significant, therefore revealing that sexual harassment and sexual assault reported by the participants was not unique in the military or out of the military. Participants who reported sexual harassment or sexual assault out of the military were the same as those who reported those experiences in the military.

Table 7

Crosstabulation of T. gondii infection status and smoking history

	Smoking Status			
T. gondii Status	No, does not smoke	Yes, currently smokes	X^2	ϕ
Negative	54 (.93)	8 (.67)	4.502**	.31
Positive	4 (.07)	4 (.33)		

NOTE: **- $p \leq .05$

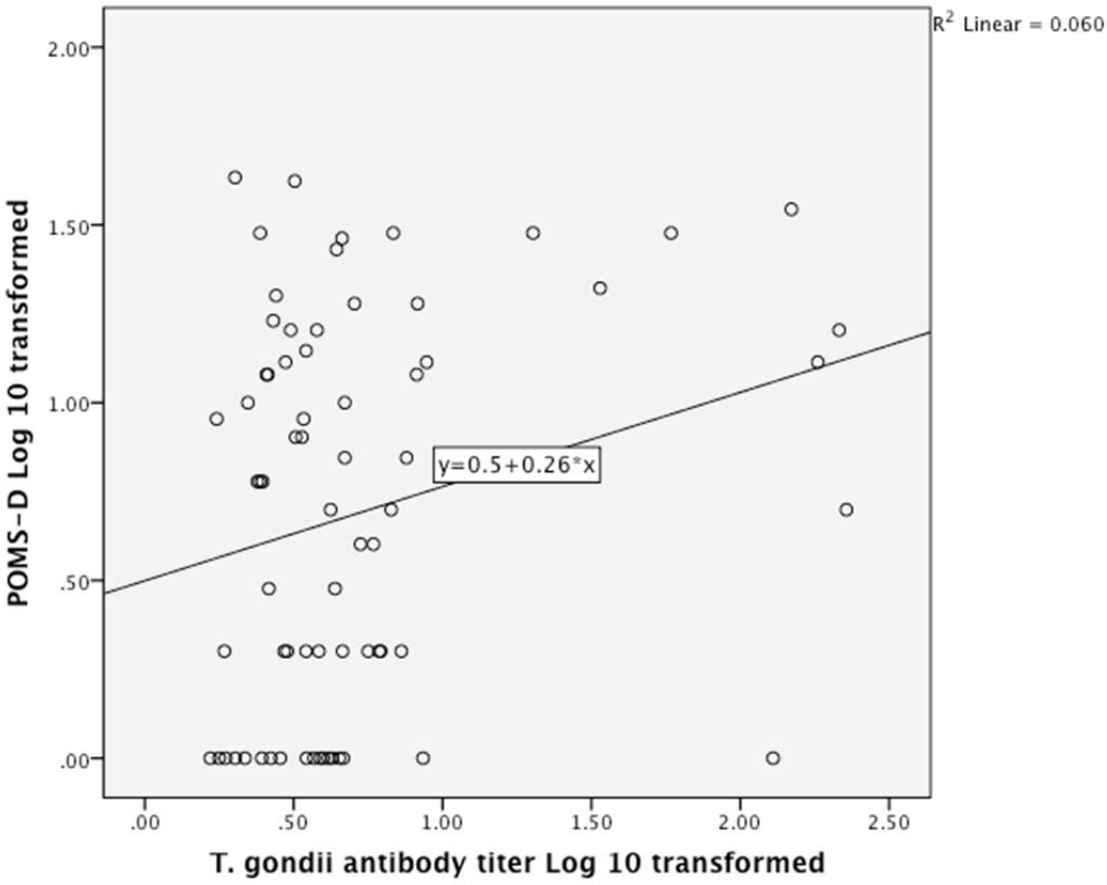


Figure 2. Scatterplot for *T. gondii* antibody titer and POMS-D

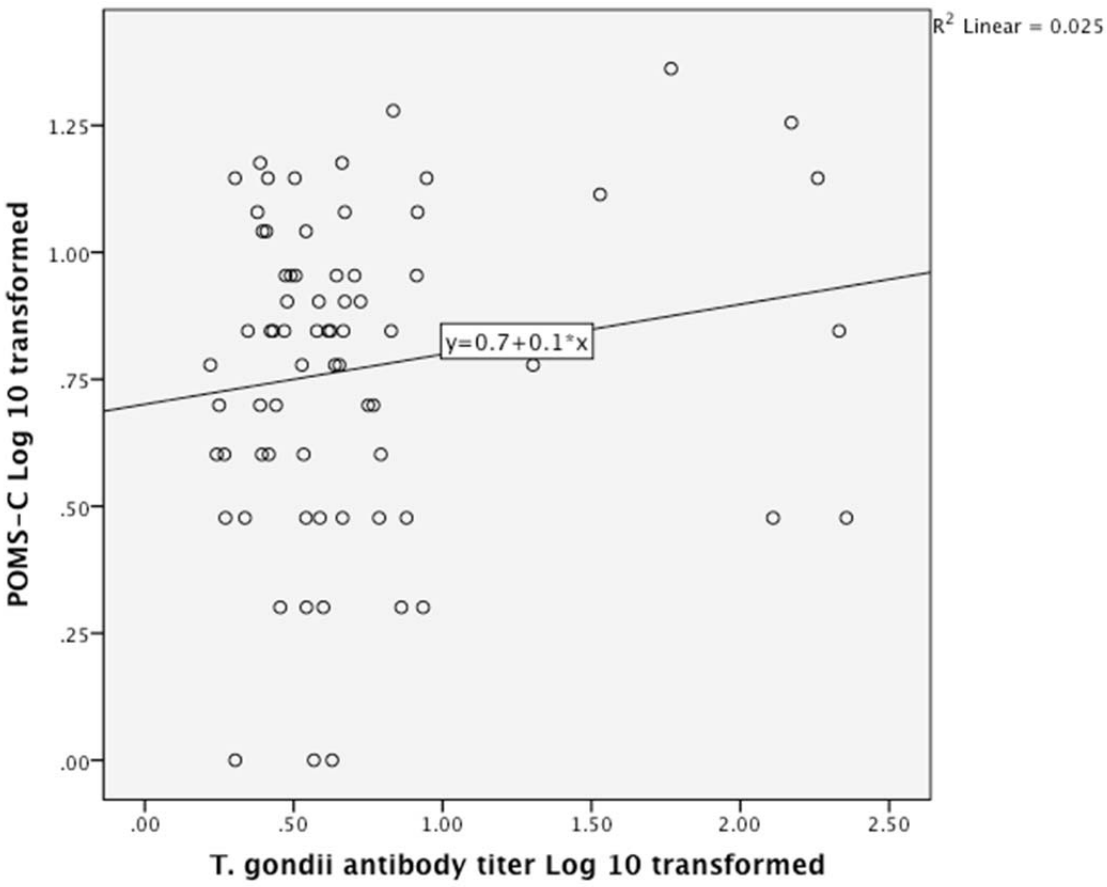


Figure 3. Scatterplot for *T. gondii* antibody titer and POMS-C

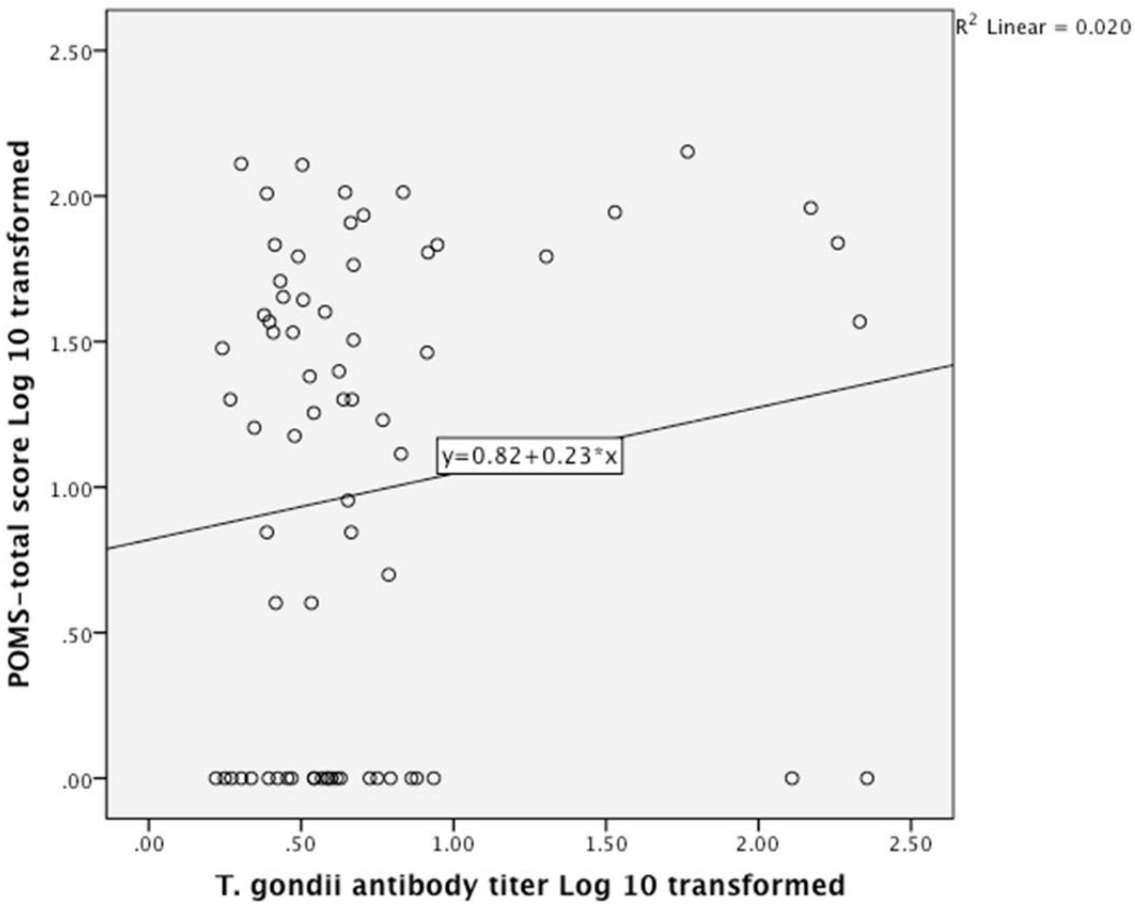
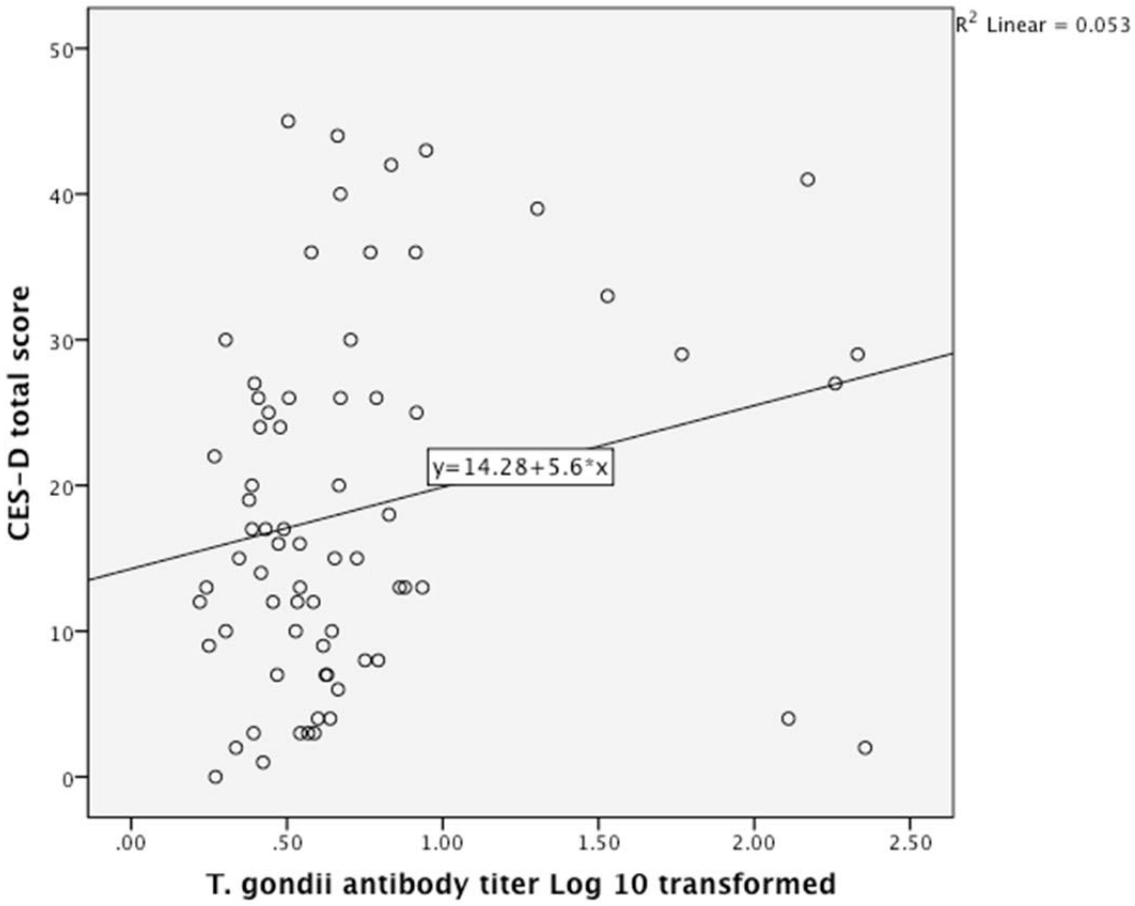


Figure 4. Scatterplot for *T. gondii* antibody titer and POMS total score



F

Figure 5. Scatterplot of *T. gondii* antibody titer and CES-D scores

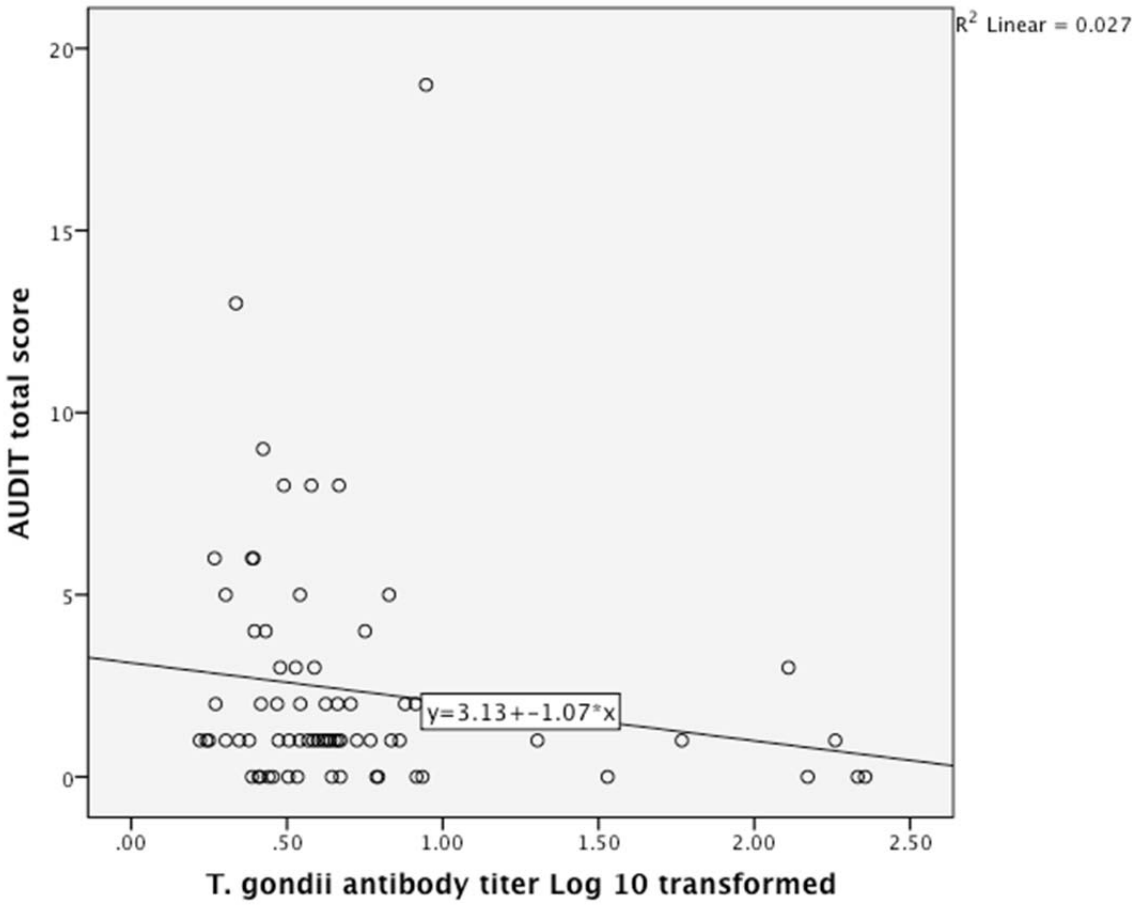


Figure 6. Scatterplot for *T. gondii* antibody titer and AUDIT score

CHAPTER FIVE

DISCUSSION

The purpose of the current study was to examine the relationships between socio-demographic variables, psychological variables, risky behaviors, and *Toxoplasma gondii* IgG antibody titer. The study was designed to answer the following question "To what extent is there a relationship between *Toxoplasma gondii* IgG antibody titers, demographics, dysphoric moods, PTSD, and risky behaviors in female veterans?" The hypothesized conceptual model proposed a relationship between socio-demographic variables (age, ethnicity, race, level of education, and marital status) and presence of *T. gondii* infection. A relationship between deployment history and *T. gondii* infection was also proposed. Finally, relationships were hypothesized between *T. gondii* infection and dysphoric moods (depressive symptoms and anxiety), post-traumatic stress symptomatology, and risky behaviors (smoking and alcohol use). Chi-square tests, Pearson's correlations, and t-tests were employed to address the aims of this study.

It is reported that of the 23 million living veterans in the United States, 2.3 million (10%) are women (US Department of Veterans Affairs, 2013). In the state of Florida, these numbers are mirrored, with approximately 164,936 female veterans out of 1.5 million living in the state (US Department of Veterans Affairs, 2013). These numbers are projected to increase, both nationwide and in the state of Florida. As the number of female veterans increases, so does the need for information regarding this population.

Toxoplasma gondii is an intracellular parasite that infects over one-third of the world's population. In the U.S., 22.5% of the population is infected by the parasite. Pappas, Roussos, and Falagas (2009), reported global prevalence rates of *T. gondii* infection by performing an extensive literature search on studies conducted between 1999 and 2008. Participants in the current study reported locations of deployments in countries such as Saudi Arabia (reported infection rate up to 50%), Kuwait (reported infection rate up to 50%), Iraq (reported infection rate approximately 50%), and Iran (reported infection rate up to 70%). When considering the U.S. infection rate as well as the infection rates in the above listed geographical locations, it was hypothesized that the infection rate in a sample of 70 female veterans would exceed that of the U.S. However, the infection rate for the sample in the current study was 11.5% (8 participants).

Dubey and Jones (2008) reported that in a population-based National Health and Examination Nutrition Study (NHANES), overall seroprevalence of *T. gondii* increased with age and was higher among non-Hispanic African Americans and Mexican Americans than non-Hispanic Caucasian Americans. In addition, Alvarado-Esquivel, Torres-Castorena, Liesenfeld, Estrada-Martinez, and Urbina-Alvarez (2012) reported that *T. gondii* infection was associated with low socio-demographic status in a sample of 133 Mexican patients. The authors reported an infection rate of 8.3% and defined low economic status as no education and low-income. In that regard, the proposed conceptual model of the current study suggested a relationship between socio-demographic variables, deployment history, and *T. gondii* antibody titer. Statistically significant relationships between *T. gondii* and ethnicity (self-report of Hispanic or not Hispanic),

marital status, level of education, and age were shown, supporting that aspect of the hypothesized conceptual model.

T. gondii infection rates in countries located in the Middle East, such as Afghanistan, Iraq, Kuwaut, and Saudi Arabia have been reported much higher than that of the U.S. It was hypothesized that there should be a relationship between female veterans that reported at least one deployment and *T. gondii* antibody titer. A relationship between deployment history and *T. gondii* infection was not statistically significant, which was an unexpected finding and does not support the conceptual model proposed in this study.

Previous studies have also shown relationships between *T. gondii* antibody titer and dysphoric mood, including depressive symptoms, anxiety, and other psychiatric disorders. Groer et al. (2011) reported a positive correlation of *T. gondii* IgG antibody titer with POMS depression and POMS anxiety subscale scores in a sample of 414 pregnant women, 44 of which were positive for *T. gondii* infection. Amminger et al. (2007) reported that in 105 participants that were high risk for psychosis, higher levels of IgG antibodies against *T. gondii* were significantly associated with more severe positive psychotic symptoms. *T. gondii* infection has also been linked to psychiatric disorders such as schizophrenia and bipolar disorder (Pearce et al., 2012).

Dysphoric mood and post-traumatic stress were key variables examined in the second aim. Scores on the Center for Epidemiologic Studies depression scale (CES-D) and Profile of Mood States (POMS), including the 6 subscales and total score, operationalized dysphoric mood. A significant positive linear relationship between *T. gondii* IgG antibody titer and 2 of the 6 subscales- depression and confusion/

bewilderment, as well as the total score was shown. Participants that were *T. gondii* positive had statistically significant higher log10 transformed POMS depression, tension, and anxiety subscale scores. In addition, though not statistically significant, a correlation was seen between *T. gondii* antibody titer and CES-D scores. The relationships between *T. gondii* antibody titer, depressive symptoms, anxiety, anger, and confusion support the conceptual model in this study. However, in regards to PTSD, the conceptual model is not supported. No studies to date have examined relationships between *T. gondii* and PTSD.

Symptomatology of PTSD was first termed post-traumatic stress disorder in the 1970s. However, manifestations of the disorder were described as early as the Greek and Roman era (Ramaswamy et al., 2005). There is a multitude of possible mechanisms to explain the symptomatology of PTSD. According to the DSM V (2013), a diagnosis of PTSD can only be made when an individual was exposed to a traumatic, catastrophic event. Patients with chronic PTSD often experience a life of remission and relapses. Misdiagnosis as depression, anxiety, or bipolar disorder may occur (Reeves, 2007). In addition, treatment modalities can vary as well. Unlike other psychiatric disorders, there is not a clear-cut option to treatment of PTSD. With so many possible causes and co-occurring problems of PTSD, a relationship between PTSD and *T. gondii* may not have been apparent in the current study.

Possible mechanisms for the relationship between *T. gondii* and depression, anxiety, and overall dysphoric mood can be explained by cyst formation in the latent stage of *T. gondii* infection. One of the most important locations for cyst development in *T. gondii* infection is in the brain. A variety of psychiatric and neurological disorders may

be attributed to alterations in the brain that occur during infection. These alterations may include immunological, pathological, anatomical, alterations in neurotransmitters and gene expression (Dalimi & Abdoli, 2012). The IDO pathway results in a decrease in serotonin in the brain of the host, possibly playing a role in depression and anxiety seen in individuals infected by the parasite (Flegr, 2013). In addition, the hippocampus and amygdala are brain structures that are associated with neurological and psychiatric diseases such as depression, schizophrenia, anxiety, and Alzheimer's disease (da Silva & Langoni, 2009); Mervaala et al., 2000; Tanskanen et al., 2005). Studies have reported an increase in cyst density, inflammatory processes, and pathological alterations in those brain structures (Gatkowska, Wieczorek, Dziadek, Dzitko, & Dlugonska, 2012; Hermes et al., 2008; Vyas et al., 2007).

The relationships between *T. gondii* and depressive symptoms, anger, confusion, and overall dysphoric moods that were found in this current study are particularly important because it has been reported that individuals infected by *T. gondii* have a higher risk of suicide. In a study conducted by Arling et al. (2009), 218 participants with a history of recurrent mood disorders (119 with a history of suicide attempt and 99 without a history of suicide attempt), it was reported that a significantly higher *T. gondii* IgG antibody titers were seen in suicide attempters when compared to participants with no history of suicide attempt. In Turkey, Yagmur, Yazar, Temel, and Cavusoglu (2010) conducted a study to examine a relationship between *T. gondii* and suicide attempts in a sample of 200 individuals that attempted suicide and 200 individuals that did not attempt suicide and reported that the *T. gondii* antibody titer was significantly higher in the

individuals who had attempted suicide than in the control group. This relationship is troubling, particularly in a group, such a veterans, that have an increased risk for suicide.

In April 2013, the U.S. Department of Defense released information regarding suicide data for the Army. In 2012, there were 297 confirmed suicides in both active duty soldiers and the reserves and 27 potential suicides that are under investigation. Just in the months of January to April in 2013, there have been 109 potential suicides, 43 have been confirmed and 66 are under investigation. With the number of suicides increasing annually across all branches of the military, both in active duty soldiers and in the reserves, there is a necessity to identify possible factors related to suicide and suicide attempts, including infection by *T. gondii*.

Relationships between *T. gondii* titer and risky behaviors were examined in the third aim. As discussed above, previous studies have reported that the hippocampus and amygdala are affected by infection of *T. gondii.* Along with a variety of neurologic and psychiatric disorders, these brain structures are also involved in non-conditioned anxiety and defense behaviors. The behavioral changes reported in previous studies in individuals with a positive *T. gondii* titer provided the framework for this aim and are operationalized by smoking status, scores on the alcohol use disorder identification tool (AUDIT), and report a history of sexual assault/ harassment, both in the military and out of the military.

Risk-taking behaviors have been examined in military samples. Combat exposure and deployment history have been reported as predictors for engaging in risk-taking behaviors (Kelley et al., 2012; Killgore et al., 2008; Thomsen, Stander, McWhorter, Rabenhorst, & Milner, 2011). As expected, a statistically significant relationship between positive *T. gondii* titer and smoking status was shown in the current study. A study

conducted by Klesges, Sherrill-Mittleman, Ebbert, Talcott, and Debon (2010) sought to assess tobacco use in a sample of 5225 Air Force airmen. The authors reported that after a period of tobacco abstinence (6 weeks with no tobacco), an escalation in tobacco use after the abstinence period was related to participants who were engaging in risk-taking behaviors, including alcohol use. However, the negative relationship between *T. gondii* status and alcohol use was unexpected. The relationship indicates that as *T. gondii* titer increases, alcohol use decreases. Wilk et al. (2010) conducted a study to examine the relationship of combat experiences and alcohol misuse in 1120 U.S. soldiers. The authors reported that 25% (N=275) of the participants screened positive for alcohol misuse. The current study showed 8% of the participants scoring above the cut-off to indicate hazardous alcohol use, far less than what has been reported in other studies. The negative relationship between these 2 variables in the current study does not support the conceptual framework proposed, nor does the lack of relationships between a history of sexual assault or harassment with *T. gondii* antibody titer.

Gender differences have been reported both in *T. gondii* infection and risk-taking in the military. Lindova et al. (2006) conducted a study to examine gender differences in behavioral changes induced by latent toxoplasmosis in a sample of 72 uninfected men, 142 uninfected women, 20 infected men, and 29 infected women. Gender differences were seen in infected participants and uninfected participants. The authors reported significant differences between gender in regards to mistrust (vigilance), relationship (warmth), self-control (rule consciousness), and clothes tidiness. In addition, infected women's scores on mistrust and self-consciousness were higher than uninfected women. Gender differences have also been reported in risk-taking behaviors in samples of

military veterans. In a study conducted by Maguen et al. (2012) with a large sample of 7251 active duty soldiers (6697 men and 554 women), men were more likely to report hazardous alcohol use. Twelve percent of women and 1% of men reported military sexual trauma in war zones. These gender differences are important to note, as a majority of previous studies focused on male soldiers, with women being underrepresented. Gender was not examined in this study, however, it is important to consider for future studies.

A possible explanation for the lack of relationships between *T. gondii* antibody titer and history of sexual harassment or assault is that risky behaviors of a sexual nature include acts such as engaging in unprotected sex or with multiple partners. Data was collected from a primary study in which history of sexual harassment or assault was obtained, not engagement of risky sexual behaviors. Future studies should include questionnaires regarding risk-taking behaviors.

Limitations

Small sample size is a limitation of this study. Therefore, a larger sample size recruited from a more extensive geographical area is needed to add additional power to the study as well as expand the generalizability of the results. Also, the study design is cross-sectional and exploratory and therefore, causation can be examined. Also, the instruments used in this study are screening tools, not to be used for diagnostic purposes. Questionnaires were self-report. Participants were not required to answer all items in the questionnaires, causing potential for missing data in the data set. The population of interest was that of female veterans and recruitment of these participants proved difficult. Female veterans were difficult to identify and contact. A possible reason for this difficulty is that there are female veterans do not identify themselves as veterans, whether

it was because they were not deployed to combat areas or feel that they are not included in this group as they are not men (Guarino, 2010). A few participants decided to withdraw from the study due to concerns they had pertaining to data management and confidentiality. The research team was diligent in thoroughly explaining the informed consent, which addressed these issues. In regards to sample size, an additional limitation was the low number of female veterans with a positive *T. gondii* antibody status. Unfortunately, this is a finding that could not be addressed until after data was collected and blood samples were analyzed. It is possible that the frequency is underrepresented due to the ELISA kits being somewhat imprecise in the lower titer range.

The current study was designed after data collection began. Therefore, in regards to predictors and risk factors that have been previously reported in regards to *T. gondii* infection, additional information could have been obtained (i.e., history of owning cats, likeliness to consume raw meat, etc.). It is thought that *T. gondii* antibody titer decreases with time, as more time elapses between initial infection and time of recruitment into the study, there is likely a decrease in antibody titer (Flegr, Kodym, & Tolarova, 2000). As the mean age of the sample is almost 50 years of age, infection may have occurred years ago. Future studies could include a sample of female veterans that were recently deployed to the Middle East. Also, in order to measure *T. gondii* antibody titer, an Enzyme-Linked Immunosorbent Assay (ELISA) is employed. There are multiple manufacturers without a gold standard for which kit is preferred. However, the specificity and sensitivity of the kits may vary from one manufacturer to another (CDC, 2009). In this study, blood samples were analyzed on 2 separate ELISA kits from 2 different manufacturers in order to compare the antibody titers for the entire sample.

Finally, when data was collected, there was no requirement to the items having to be completed, which contributes to the issue of missing data. Missing data was an issue that was dealt with and discussed in Chapter 4, but is a limitation nonetheless. The POMS scale, CES-D scale, and PCL-M scale are all screening tools for their respective symptomatology, not diagnostic.

Conclusion

As more female veterans are returning home from combat or entering the healthcare system for the first time, additional research is needed in order to address the mental and physical needs of this population. *T. gondii* is a parasite that infects over one-third of the population, including 22% of the U.S. population. Previous research has suggested a relationship between *T. gondii* infection and various psychosocial issues, such as behavior and personality changes, as well as psychiatric disorders. Due to the trauma experienced by female veterans, this vulnerable population is also at an increased risk for developing similar changes and disorders. Therefore, examining relationships between *T. gondii* infection and various psychosocial variables in female veterans is an important issue to consider. No study to date has examined these relationships. The current study sought to examine the extent to which there are relationships between *T. gondii* infection and demographic, psychosocial, and risky behaviors. Results of the current study show relationships between *T. gondii* infection and demographic variables, psychosocial variables, and risky behaviors in a sample of female veterans and require additional research.

Implications for Future Research

To perform analyses on data in order to examine the predictors of *T. gondii*, a larger sample of *T. gondii* positive participants is needed including larger sample sizes positive for *T. gondii* infection. In addition, longitudinal studies should be conducted in order to determine when *T. gondii* infection occurred and seek information regarding severity of infection and the variables included in this current study in a sample of female veterans. Non-military controls should be employed when conducting future research.

The current study encountered an issue in regards to the reliability of the *T. gondii* antibody titer. In subsequent studies, the researcher should produce a *T. gondii* ELISA to analyze antibody titers. With the production of an ELISA plate, rather than purchasing a kit from a manufacturer, the results may be found to be more reliable. Also, samples of female veterans should be recently deployed, possibly with blood samples collected prior to deployment and after returning from deployment. Additional questionnaires should be included in future studies, such as Evaluation of Risk Questionnaire (EVAR), the Driving Behavior Questionnaire, and the Iowa Gambling Task. These questionnaires will further assess risk-taking behaviors. Examination of reaction times should also be implemented in future studies. It has been reported that *T. gondii* antibody titer was associated with delayed reaction time and therefore is an important variable to assess in the military. Finally, suicide in the military is currently an issue that is receiving attention. There is an increasing number of suicide and suicide attempts and a reported relationship between suicide and *T. gondii.* Future studies should include variables regarding suicidality and possible relationships with T. gondii antibody titer in sample of soldiers and military veterans.

The U.S. has been in war conflict for over a decade and military veterans are returning from areas of war having experienced trauma that is in need of immediate attention. In order to properly treat veterans, both male and female, providers must be aware of possible issues that may be experienced by this specific population. The variables included in this study should be used in future studies, with the addition of variables that may be predictors of *T. gondii* infection.

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APPENDICES

Appendix A
Demographics Form

Last Name:
First Name:

Street Address:
City:
State:
Zip:
Phone # 1:
Phone #2:
Email Address:
Secondary Contact Name:
Phone:

Current Age:

Ethnic Background:

1) Caucasian
2) African American
3) Asian/ Pacific Islander
4) Eskmio/ Native American Indian
5) Mixed (please specify): _____
6) Other (please specify): _____

Do you consider yourself to be Hispanic?

Yes
No

Work status: (circle one number only)

1) Unemployed
    a. Disabled
    b. Retired
    c. Supported by other
2) Employed full time
    Hours/ week
3) Employed part-time
    Hours/ week
4) On leave
    a. with pay
    b. without pay

Level of education

1) Less than high school
2) High school graduate? GED

3) Vocational/ Technical degree beyond high school
4) Some college (incomplete)
5) Community college degree (AA/AS)
6) College/ university degree (BA/BS
7) Masters degree
8) Doctoral degree

Current Living Arrangement

1) Live alone
2) Live with parents
3) Live with roommate with is not spouse/ partner
4) Live with spouse/ partner
5) Live with spouse/ partner and children
6) Live with children (no spouse/ partner)
7) Other: (Specify)_____

Marital status: (Circle one number only)

1) Single/ never married
2) Married
3) Separated
4) Divorced
5) Widowed

Number of roles you engage in: (Check all that apply)

1) Mother
2) Spouse/ Partner
3) Caregiver
4) Worker
5) Student
6) Daughter
7) Other role (specify): _____

Household income: (Circle one number only)

1) under $4,999
2) $5,000- $14,999
3) $15,000- $24,999
4) $25,000- $39,999
5) $40,000- $69,999
6) $70,000+

Branch of the service (Circle all the apply; Check active or reserves)

1) Army (_____Active Duty _____Reserves)
2) Navy (_____Active Duty _____Reserves)
3) Air Force (_____Active Duty _____Reserves)

4) Marine Corps (_____Active Duty _____Reserves)
5) Coast Guard (_____Active Duty _____Reserves)
6) National Guard (_____Active Duty _____Reserves)

Total Years of Service: _____ years

Highest Rank Attained: _____

Did you complete a deployment?
Yes
No

Deployment History #1 N/A
Dates of Deployment (mm/dd/yy): From: To:
Location of Deployment (City, State, Country): _____
Deployment Role/ Assignment: _____

Deployment History #2 N/A
Dates of Deployment (mm/dd/yy): From: To:
Location of Deployment (City, State, Country): _____
Deployment Role/ Assignment: _____

Deployment History #3 N/A
Dates of Deployment (mm/dd/yy): From: To:
Location of Deployment (City, State, Country): _____
Deployment Role/ Assignment: _____

Deployment History #4 N/A
Dates of Deployment (mm/dd/yy): From: To:
Location of Deployment (City, State, Country): _____
Deployment Role/ Assignment: _____

Deployment History #5 N/A
Dates of Deployment (mm/dd/yy): From: To:
Location of Deployment (City, State, Country): _____
Deployment Role/ Assignment: _____

19. Health History

Directions: Circle the number of each condition that you have been diagnosed or treated for at any time (past or present). Write in the year that you were first diagnosed.

	Condition or Diagnosis	**Year Diagnosed (First occurrence)**

[1]	High blood pressure	
[2]	Coronary Heart Disease	
[3]	Heart Attack	
[4]	Stroke, cerebrovascular accident (CVA), blood clot or bleeding in the brain, or transient ischemic attack (TIA)	
[5]	Heart Valve Disease	
[6]	Peripheral Vascular Disease	
[7]	Asthma	
[8]	Emphysema, chronic bronchitis, or chronic obstructive lung disease	
[9]	Stomach ulcers or peptic ulcers	
[10]	Gastrointestinal disorders If yes, please identify condition(s):	
[11]	Autoimmune Disease If yes, please identify condition(s):	
[12]	Diabetes	
[13]	Problems with your kidneys or kidney disease	
[14]	Recurrent urinary tract infections (UTIs)	
[15]	Cirrhosis or liver disease	
[16]	Thyroid Disease	
[17]	Arthritis	
[18]	Recurrent skin rashes	
[19]	Osteoporosis	
[20]	Cancer If yes, please identify condition(s):	
[21]	Ringing in your ears	
[22]	Severe headaches or migraines	
[23]	Menopause	
[24]	Reproductive disorders or menstrual irregularities If yes, please identify condition(s):	

[25]	Depression	
[26]	Anxiety	
[27]	Psychiatric Disorder If yes, please identify condition(s):	
[28]	Allergies If yes, please describe:	

Surgical History

Please identify the reasons for your last 5 surgeries. Mark N/A if you have not had any surgeries.

20. Surgical History #1: ☐ N/A

20a. Date of Surgery (mm/dd/yy): _____ / _____ / _____

20b. Reason for surgery: _____

21. Surgical History #2: ☐ N/A

21a. Date of Surgery (mm/dd/yy): _____ / _____ / _____

21b. Reason for surgery: _____

22. Surgical History #3: ☐ N/A

22a. Date of Surgery (mm/dd/yy): _____ / _____ / _____

22b. Reason for surgery: _____

23. Surgical History #4: ☐ N/A

23a. Date of Surgery (mm/dd/yy): _____ / _____ / _____

23b. Reason for surgery: _____

24. Surgical History #5: ☐ N/A

24a. Date of Surgery (mm/dd/yy): _____ / _____ / _____

24b. Reason for surgery: _____

Current Medications

25. Are you currently taking any medications?

[1] Yes

[2] No

	Name of Medication	Reason for taking	Frequency of Use (i.e. daily or as needed)
25a.			
25b.			
25c.			
25d.			
25e.			
25f.			
25g.			
25h.			
25i.			
25j.			

Pregnancy History

26. Have you ever been pregnant?

[1] Yes
[2] No

26a. Total number of pregnancies: _____

26b. Number of full term births: _____

26c. Number of preterm deliveries: _____

26d. Number of abortions: _____

26e. Number of miscarriages: _____

26f. Number of ectopic pregnancies: _____

26g. Number of living children: _____

26h. Disorders of pregnancy (circle all that apply)

[1] Preterm labor/birth
[2] Gestational hypertension/Preeclampsia/Eclampsia
[3] Gestational diabetes
[4] Spontaneous Premature Rupture of Membranes
[5] Recurrent pregnancy loss
[6] Child with a genetic or chromosomal defect
[7] Other: (Please specify) _____

Smoking History

27. Do you smoke cigarettes?

[1] Yes, currently
[2] No, previously [2.1] Quit date (mm/yy): _____ / _____
[3] No, never

27a. Total years smoked _____

27b. Number of packs/day_____

27c. Do you live with a smoker?
[1] Yes
[2] No

Diet

In an average day, …

28a. How many cups of coffee or tea do you drink? _____

28b. How many soft drinks/sodas do you drink? _____

28c. How many servings of fruits do you eat? _____

28e. How many servings of vegetables do you eat? _____

Exercise/Activity Level

29a. How many days a week do you exercise? _____

29b. On average, how many minutes a day do you exercise? _____

29c. Describe the type of exercise you complete:
[1] Not strenuous (light housework)
[2] Mildly strenuous (walking your pet)
[3] Somewhat strenuous (walking up hill)
[4] Very strenuous (jogging)

29d. Which option below best describes your current level of physical activity?

(Circle <u>one</u> number only)
[1] Fully active, able to carry on all usual activities without restriction
[2] Restricted in physically strenuous activity, but can walk and able to carry out
light housework.
[3] Can walk and take care of yourself, but unable to carry out any work activities;
up more than half a day.
[4] Need some help taking care of yourself; spend more than half a day in bed or a
chair.
[5] Cannot take care of yourself at all; spend all of time in bed or chair.

Appendix B
Center for Epidemiologic Studies Depression (CES-D) Scale

		During the past week:			
		Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1	*I was bothered by things that usually don't bother me.*	0	1	2	3
2	*I did not feel like eating; my appetite was poor.*	0	1	2	3
3	*I felt that I could not shake off the blues even with help from my family or friends.*	0	1	2	3
4	*I felt that I was just as good as other people.*	3	2	1	0
5	*I had trouble keeping my mind on what I was doing.*	0	1	2	3
6	*I felt depressed.*	0	1	2	3
7	*I felt that everything I did was an effort.*	0	1	2	3
8	*I felt hopeful about the future.*	3	2	1	0
9	*I thought my life had been a failure.*	0	1	2	3
10	*I felt fearful.*	0	1	2	3
11	*My sleep was restless.*	0	1	2	3
12	*I was happy.*	3	2	1	0
13	*I talked less than usual.*	0	1	2	3
14	*I felt lonely.*	0	1	2	3
15	*People were unfriendly.*	0	1	2	3
16	*I enjoyed life.*	3	2	1	0
17	*I had crying spells.*	0	1	2	3
18	*I felt sad.*	0	1	2	3
19	*I felt that people disliked me.*	0	1	2	3
20	*I could not get "going."*	0	1	2	3

Appendix C
Profile of Mood States (POMS)

Profile of Mood States
Subject's Initials _____ Birth date _____
Date _____
Subject Code No. _____
Directions: Describe HOW YOU FEEL RIGHT NOW by checking one space after each of the words listed below:

FEELING	Not at all	A little	Mod.	Quite a bit	Extremely
Friendly	1	2	3	4	5
Tense	1	2	3	4	5
Angry	1	2	3	4	5
Worn Out	1	2	3	4	5
Unhappy	1	2	3	4	5
Clear-headed	1	2	3	4	5
Lively	1	2	3	4	5
Confused	1	2	3	4	5
Sorry for things done	1	2	3	4	5
Shaky	1	2	3	4	5
Listless	1	2	3	4	5
Peeved	1	2	3	4	5
Considerate	1	2	3	4	5
Sad	1	2	3	4	5
Active	1	2	3	4	5
On edge	1	2	3	4	5
Grouchy	1	2	3	4	5
Blue	1	2	3	4	5
Energetic	1	2	3	4	5
Panicky	1	2	3	4	5
Hopeless	1	2	3	4	5
Relaxed	1	2	3	4	5
Unworthy	1	2	3	4	5
Spiteful	1	2	3	4	5
Sympathetic	1	2	3	4	5
Uneasy	1	2	3	4	5
Restless	1	2	3	4	5
Unable to concentrate	1	2	3	4	5
Fatigued	1	2	3	4	5
Helpful	1	2	3	4	5
Annoyed	1	2	3	4	5
Discouraged	1	2	3	4	5
Resentful	1	2	3	4	5

Nervous	1	2	3	4	5
Lonely	1	2	3	4	5
Miserable	1	2	3	4	5
Muddled	1	2	3	4	5
Cheerful	1	2	3	4	5
Bitter	1	2	3	4	5
Exhausted	1	2	3	4	5
Anxious	1	2	3	4	5
Ready to fight	1	2	3	4	5
Good-natured	1	2	3	4	5
Gloomy	1	2	3	4	5
Desperate	1	2	3	4	5
Sluggish	1	2	3	4	5
Rebellious	1	2	3	4	5
Helpless	1	2	3	4	5
Weary	1	2	3	4	5
Bewildered	1	2	3	4	5
Alert	1	2	3	4	5
Deceived	1	2	3	4	5
Furious	1	2	3	4	5
Effacious	1	2	3	4	5
Trusting	1	2	3	4	5
Fullofpep	1	2	3	4	5
Bad-tempered	1	2	3	4	5
Worthless	1	2	3	4	5
Forgetful	1	2	3	4	5
Carefree	1	2	3	4	5
Terrified	1	2	3	4	5
Guilty	1	2	3	4	5
Vigorous	1	2	3	4	5
Uncertain about things	1	2	3	4	5
Bushed	1	2	3	4	5

Appendix D
PTSD Checklist- Military (PCL-M) version

PTSD CheckList – Military Version (PCL-M)

Patient's Name: _____

Instruction to patient: Below is a list of problems and complaints that veterans sometimes have in response to stressful life experiences. Please read each one carefully, put an "X" in the box to indicate how much you have been bothered by that problem *in the last month*.

No.	Response	Not at all (1)	A little bit (2)	Moderatly (3)	Quite a bit (4)	Extremely (5)
1.	Repeated, disturbing *memories, thoughts, or images* of a stressful military experience from the past?					
2.	Repeated, disturbing *dreams* of a stressful military experience from the past?					
3.	Suddenly *acting* or *feeling* as if a stressful military experience *were happening* again (as if you were reliving it)?					
4.	Feeling *very upset* when *something reminded* you of a stressful military experience from the past?					
5.	Having *physical reactions* (e.g., heart pounding, trouble breathing, or sweating) when *something reminded* you of a stressful military experience from the past?					
6.	Avoid *thinking about* or *talking about* a stressful military experience from the past or avoid *having feelings* related to it?					
7.	Avoid *activities* or *situations* because they *remind you* of a stressful military experience from the past?					
8.	Trouble *remembering important parts* of a stressful military experience from the past?					
9.	Loss of *interest in things that you used to enjoy?*					
10.	Feeling *distant* or *cut* off from other people?					
11.	Feeling *emotionally numb* or being unable to have loving feelings for those close to you?					
12.	Feeling as if your *future* will somehow be *cut short*?					
13.	Trouble *falling* or *staying asleep*?					
14.	Feeling *irritable* or having *angry outbursts*?					
15.	Having *difficulty concentrating*?					
16.	Being *"super alert"* or watchful on guard?					
17.	Feeling *jumpy* or easily startled?					

PCL-M for DSM-IV (11/1/94) Weathers, Litz, Huska, & Keane National Center for PTSD - Behavioral Science Division

Appendix E
Alcohol Use Disorder Identification Test (AUDIT)

The Alcohol Use Disorders Identification Test: Self-Report Version

PATIENT: Because alcohol use can affect your health and can interfere with certain medications and treatments, it is important that we ask some questions about your use of alcohol. Your answers will remain confidential so please be honest. Place an X in one box that best describes your answer to each question.

Questions	**0**	**1**	**2**	**3**	**4**
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more
3. How often do you have six or more drinks on one occasion ?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session ?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
7. Howoftenduringthelastyear have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
8. How often during the last year have you been unable to remem- ber what happened the night before because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
9. Have you or someone else been injured because of your drinking?	No		Yes, but not in the last year		Yes, during the last year
10.Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year
					Total

Appendix F
Sexual Harassment and Assault information

1. Have you experienced sexual harassment (made to feel inferior, ridiculed for your gender, denied job opportunities) in your life outside the military?

Yes ……..
No……..
If yes, how often?
Very frequently…. Frequently….. Occasionally….. Seldom…… Very seldom……..

2. Have you experienced sexual harassment (made to feel inferior, ridiculed for your gender, denied job opportunities) in your life in the military?

   Yes ……..
   No……..
If yes, how often?
Very frequently…. Frequently….. Occasionally….. Seldom…… Very seldom…
……..

3. Have you experienced sexual assault (rape, attempted rape) in your life outside the military?

   Yes……
   No……..
If yes, how often?
Very frequently…. Frequently….. Occasionally….. Seldom…… Very seldom……..

If yes, did this occur in childhood?
Very frequently…. …. Frequently….. Occasionally….. Seldom…… Very seldom……..
Never…….

4. Have you experienced sexual assault (rape, attempted rape) in your life in the military?
5.

   Yes……..
   No……..
If yes, how often?
Very frequently…….. Frequently….. … Occasionally……. Seldom…… Very seldom……..