***Toxoplasma gondii* Seroprevalence: 30 Year Trend in an HIV-infected U.S. Military Cohort**

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Abstract

To determine if *Toxoplasma gondii* IgG antibody prevalence is declining in HIV-infected persons, we analyzed data (1984-2013) from the U.S. Military HIV Natural History Study. We found that *T. gondii* seroprevalence at enrollment was associated with age and decreased significantly after 1995 ($p=0.004$), similar to the general U.S. population.

Key words: *Toxoplasma gondii*, antibody, prevalence, trend, human immunodeficiency virus,

Toxoplasma gondii may cause encephalitis and other life-threatening illnesses in the late stages of human immunodeficiency virus (HIV) disease [1]. In the United States, HIV-related toxoplasmosis has markedly declined in incidence, hospitalizations, and deaths since 1995, coinciding with the availability of highly active antiretroviral treatment [2-5]. However, approximately 3,000 toxoplasmosis hospitalizations occurred among HIV-infected patients in 2008, indicating that significant burden of disease is still present [3]. In the general U.S. population, the prevalence of *T. gondii* antibodies has been declining over the past two decades [6]. However, data specifically demonstrating such a decline among persons infected with HIV are lacking. We examined data from a cohort of HIV-infected military personnel screened for *T. gondii* IgG antibody over a 30-year period.

The U.S. Military HIV Natural History Study (NHS) is an ongoing, continuous enrollment observational cohort of Department of Defense active duty military members and beneficiaries diagnosed with HIV infection. Participants are followed at five military medical centers in the United States as previously described [7]. Due to regular HIV screening, the military members are generally healthy and history of intravenous drug use is rare [8]. Enrolling since 1986, the NHS has approximately 5,700 participants with signed written consent. Approval for this research was obtained centrally and from the institutional review boards at each participating site. Following enrollment, subjects have study visits approximately every 6-12 months. Data collected at each visit include demographic information, past and interim medical histories, medications, vaccinations, and clinical laboratory studies. IgG antibodies to *T. gondii* are determined as part of a baseline evaluation at enrollment.

We analyzed a subset of NHS subjects evaluated at San Antonio Military Medical Center (SAMMC) at Fort Sam Houston, Texas. Data included *T. gondii* IgG antibody, date of serum

collection and demographic variables. Subjects with indeterminate *T.gondii* antibody results after repeat testing were excluded from the analysis. We chose data from a single site because SAMMC was one of the earliest study sites to participate in the NHS and had demonstrated internal consistency in *T. gondii* antibody assay over three decades. A hospital laboratory based enzyme-linked immunosorbent assay (ELISA) was used during the study period with the exception of two years (1986-1988) in which indirect fluorescent assay was utilized. After each change of method or manufacturer, the validation of the new assay was verified using correlation procedures in accordance with the standards of the College of American Pathologists [9]. Typically, assays of 100 patient samples and standard controls were found to be in agreement before the new assay was considered valid. Statistical analysis was performed using SPSS version 19.0 (IBM SPSS, Armonk, NY).

1,645 participants underwent screening for *T. gondii* IgG antibody at SAMMC from 1984 to 2013. Overall, IgG antibody was identified in 223 (13.6%) individuals (Table 1). Seropositivity was strongly associated with age ($p=0.001$) and did not significantly differ by race or gender. The prevalence was highest from 1984 to 1995 (15.0%) then declined from 1996 to 2013 (10.5%); (OR=1.50, 95% CI, 1.08-2.06; $p=0.014$). This remained significant when adjusted for age, gender, and race (OR= 1.63, 95% CI, 1.17-2.27, $p=0.004$). The proportion of participants with the antibody decreased in all age tertile groups, Caucasians, and African Americans but remained unchanged among Hispanics.

Characteristics of each decade of the study period were examined (Table 2). Over time, the cohort became older when initially screened for *T. gondii* IgG antibody with a higher proportion of Hispanic and other minority participants. Using a logistic regression multivariable model, a positive antibody test result was associated with third tertile of age (≥ 34 years) (OR=

1 1.87, 95% CI, 1.30-2.69, $p=0.001$) and less likely to occur during 2004-2013 (OR= 0.66, 95%
2 CI, 0.44-1.00, $p=0.047$), using the 1984-1993 decade as the referent.

3 *T. gondii* infects humans and a variety of animals worldwide, with cats as definitive hosts
4 [1]. In the U.S., the Centers for Disease Control (CDC) and Prevention estimated that one
5 million persons are infected with *T. gondii* each year [10]. However, according to data from the
6 National Health and Nutrition Examination Survey (NHANES), the age-adjusted seroprevalence
7 among U.S. born persons between ages of 12 to 49 years decreased from 14.1% in NHANES III
8 (1988-1994) to 9.0% in NHANES 1999-2004 to 6.7% in NHANES 2009-2010 [6]. Proposed
9 explanations for the decline in *T. gondii* seropositivity include public health education, improved
10 hygiene related to soil and cat feces, changes in meat preparation and freezing practices, and use
11 of enhanced salt solutions in meat [6]. Our observation of a significant decline in the presence of
12 *T. gondii* IgG antibody in HIV-infected military personnel suggests the benefits of such measures
13 have reached a potentially vulnerable population.

14 The strength of our study is that longitudinal data was collected over a period of time
15 spanning thirty years. Laboratory consistency was remarkable during this time with few
16 methodological changes. Our cohort is ethnically diverse, although women are under-
17 represented. Place of birth and childhood poverty were not available in our data set. *T. gondii*
18 antibody prevalence may vary among U.S. Census Bureau regions with positive rates highest in
19 the East South Central and Mid-Atlantic states and lowest in the Mountain and West North
20 Central regions [11]. Military personnel come from all states and assignment to Fort Sam
21 Houston is not related to origin of residence. The demographics of our cohort are similar to
22 HIV-infected men who have sex with men in the U.S [12]. It is significant that our cohort
23 increased in age and in proportion of Hispanics and other minorities with time. These trends

would expect to result in an increased number of positive antibody tests. Thus, changing demographics does not support the decrease in *T.gondii* seroprevalence observed in our study.

In conclusion, our findings indicate that *T.gondii* IgG antibody prevalence has declined among HIV-infected persons in the U.S. over the past 20 years, similar to the general population. It is encouraging that less exposure may contribute to fewer cases of serious toxoplasmosis in the future. However, significant reduction in rates of toxoplasmosis disease in HIV-infected persons will require a continued emphasis on HIV screening, access to healthcare, and early initiation of antiretroviral therapy.

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Table 1

Characteristics of participants tested for *Toxoplasma gondii* IgG antibody at Fort Sam Houston from 1984 to 2013 in the U.S. Military HIV Natural History Study

	<i>T. gondii</i> IgG Antibody Positive N=223	<i>T. gondii</i> IgG Antibody Negative N=1,422	P value
Median Age (IQR)	31 (25, 35)	29 (26, 39)	<0.001 ¹
Male Gender	211 (94.6%)	1,344 (93.8%)	NS ²
Ethnicity			NS ²
Caucasian	119 (53.4%)	726 (51.1%)	
African American	74 (33.2%)	521 (36.6%)	
Hispanic	22 (9.9%)	111 (7.8%)	
Other	8 (3.6%)	64 (4.5%)	
Year tested			0.014 ²
1984-1995	167 (74.9%)	947 (66.6%)	
1996-2013	56 (25.1%)	475 (33.4%)	

¹Mann-Whitney *U* test; ²Chi-square test; IQR = Interquartile Range

Table 2

Comparison of characteristics of participants in the U.S. Military HIV Natural History Study by decade of initial screen for *T. gondii* IgG antibody at Fort Sam Houston, TX.

	1984-1993 N=1,009	1994-2003 N=332	2004-2013 N=304	P value
<i>T. gondii</i> IgG Antibody positive	146 (14.5%)	44 (13.3%)	33 (10.9%)	0.11 ^{1,2}
Median Age (IQR)	29 (25, 34)	32 (27, 38)	34 (26,33)	<0.001 ³
Male Gender	950 (94.2%)	300 (90.4%)	293 (96.4%)	0.005 ¹
Race				<0.001 ¹
Caucasian	559 (55.4%)	164 (49.4%)	122 (40.1%)	
African American	357 (35.4%)	126 (38.0%)	112 (36.8%)	
Hispanic	64 (6.3%)	26 (7.8%)	43 (14.1%)	
Other	29 (2.9%)	16 (4.8%)	27 (8.9%)	

¹Chi-Square test; ²unadjusted for age, gender, and race ; ³Kruskal-Wallis test; IQR=Interquartile range