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Toxoplasma Gondii infection and cardiovascular mortality: sex-specific differences in a United States population-based cohort study

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Abstract

Background Although Toxoplasma gondii (T. gondii) infection has been linked to cardiac injury, the extent to which it increases the risk of cardiovascular disease (CVD) mortality remains unclear. We aimed to assess the association between T. gondii infection and CVD mortality in the United States population.

Methods This study used data from the National Health and Nutrition Examination Survey (NHANES) conducted between 2009 and 2014 to investigate the association between T. gondii infection and CVD mortality. The T. gondii infection status was determined by measuring serum T. gondii IgG antibody levels. CVD mortality outcomes were ascertained through linkage with the national mortality index records. Cox proportional hazard models were used to estimate the hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) of T. gondii infection on CVD mortality.

Results A total of 10,237 (Male, $n=5010$; Female, $n=5227$) individuals aged ≥ 20 years were included in the analysis, of which 1,632 were positive for T. gondii serum IgG antibodies. After a median follow-up of eight years, there were 312 deaths due to CVD. In multivariable-adjusted analyses, the risk of death from CVD was 40% higher in T. gondii-seropositive men compared with seronegative men (HR: 1.40; 95%CI: 1.02–1.93), but not in women (HR: 0.87; 95% CI: 0.57–1.34). These results remained consistent in further stratified and sensitivity analyses.

Conclusion In this large population-based cohort study, T. gondii infection was associated with an increased risk of CVD mortality in men, but not in women. Further studies are required to elucidate the underlying mechanisms and potential sex-specific differences in the effects of T. gondii infection on CVD mortality. Future investigations should focus on validating these results and exploring the potential implications for cardiovascular risk assessment and management.

Keywords Parasite, Toxoplasmosis, Cardiovascular disease, NHANES, Cohort study

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Background

Cardiovascular disease (CVD) remains the primary cause of mortality worldwide, causing 19 million deaths in 2019 [1]. Despite numerous interventions aimed at reducing CVD mortality, its prevalence and impact have not declined, with projections indicating that over 40% of the United States (U.S.) population will be affected by 2030 [2]. Thus, it is imperative to identify and modify the risk factors associated with CVD to reduce mortality rates.

Toxoplasma gondii (T. gondii) is a parasitic organism that can be transmitted through feline fecal contamination or undercooked meat, and exists in three main forms: oocysts, tachyzoites, and bradyzoites [3]. Oocysts are produced exclusively in members of the feline family, whereas tachyzoites are found in humans or other intermediate hosts, rapidly dividing within the intestinal epithelium after ingestion and dispersed throughout the body via lymphatic vessels, usually localizing in muscle and nerve tissues, causing a strong inflammatory response and tissue destruction [4]. This parasite is prevalent worldwide, with an estimated prevalence of 10.4% of the U.S. population aged 6 years and older [5]. Although most infected individuals with normal immune systems are asymptomatic and undiagnosed [6], the parasite can cause mechanical and immune-mediated damage, particularly in immunocompromised individuals or those with congenital infections [7]. Chronic T. gondii infection may lead to damage to vital organs, such as the kidneys, liver, and heart [8, 9]. The cardiovascular effects of T. gondii mainly manifest as myocarditis, characterized by inflammatory cell infiltration, with or without myocardial cell necrosis [10]. While often asymptomatic, T. gondii-induced myocarditis may be linked to gradual cardiac function deterioration [11]. This infection can lead to a range of cardiovascular complications, including arrhythmias and heart failure [12]. In cases of infectious myocarditis, sinus tachycardia frequently serves as a characteristic early indicator [13]. Additional cardiac irregularities observed in T. gondii infection cases encompass atrial fibrillation [14, 15], varying degrees of atrioventricular conduction disturbances [9], and bundle branch blocks [16]. Under certain conditions, these abnormalities may precipitate hemodynamic issues, potentially resulting in heart failure. Beyond its direct cardiac impact, T. gondii infection is associated with systemic inflammatory responses and lipid metabolism disorders [17]. Studies indicate that this infection stimulates increased production of nitric oxide (NO) and reactive oxygen species (ROS), thereby intensifying oxidative stress [18]. The synergistic effects of inflammation, lipid metabolism abnormalities, and oxidative stress may promote the formation and progression of atherosclerotic plaques, further elevating cardiovascular disease risks.

As part of the Neglected Tropical Diseases and Other Infectious Diseases Affecting the Heart (NETHeart) project [19], a recent review was conducted with the aim of systematically examining all available information on the cardiovascular effects of T. gondii [20]. Among the 48 publications that met the inclusion criteria, the majority were case reports (22/48), seven were retrospective studies, and five were cross-sectional studies lacking prospective designs. These studies mainly focused on immunocompromised populations, such as patients with AIDS or organ transplant recipients, and did not establish whether T. gondii infection contributed to the development or progression of CVD mortality. Thus, further prospective studies are needed to validate the association between T. gondii infection and CVD mortality, and to explore its pathogenesis and preventive treatment measures.

To enhance our understanding of the cardiac effects of T. gondii infection, we investigated the association between T. gondii infection and CVD mortality using data from the National Health and Nutrition Examination Survey (NHANES) in the U.S.

Methods

Data source and study design

The NHANES was conducted by the National Center for Health Statistics (NCHS), a division of the U.S. Centers for Disease Control and Prevention (CDC). The purpose of this national survey was to assess the health status of the entire U.S. population. Population-level estimates were generated using a multistage probability sampling method, and data were collected annually over a two-year cycle. We obtained data on the number of non-institutionalized civilians from the NHANES from 2009 to 2014. Demographic, disease details, laboratory, and questionnaire data on disease classification were collected. After excluding participants who were pregnant at baseline ($n=156$), under 20 years of age ($n=5192$), had cancer at baseline ($n=1360$), lacked T. gondii antibody data ($n=6$), had missing covariates ($n=2413$), or had missing death status data ($n=14$), 10,237 participants (Male, $n=5010$; Female, $n=5227$) were included in our analysis (Fig. 1). The Institutional Review Board of the CDC National Center for Health Statistics has authorized the NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm) to provide access to the research data to the public. In the NHANES study, all participants provided informed written consent upon enrollment. The study followed the principles outlined in the Declaration of Helsinki (revised in 2013) and received approval from the Ethics Committee of The Second Affiliated Hospital of Gannan Medical University (EFRJ20230214001).

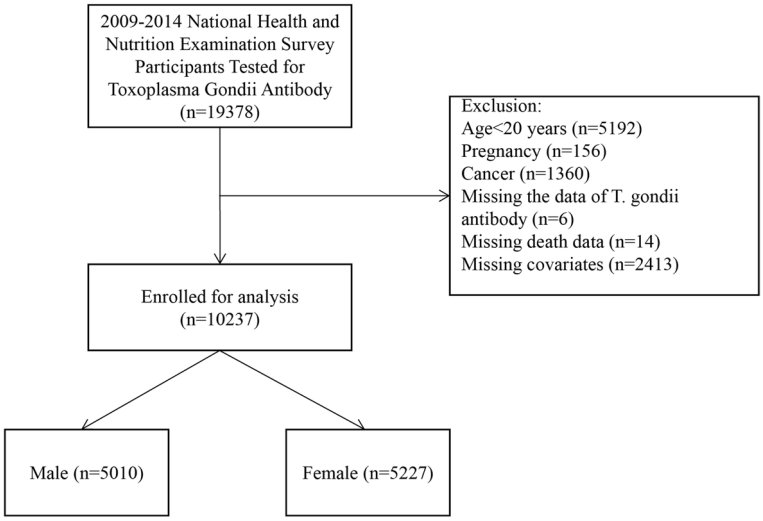


Fig. 1 Study flowchart

Definition of T. Gondii infection

T. gondii IgG antibodies were measured using the Toxoplasma IgG EIA (Bio-Rad, Redmond, WA, USA). Results ≥ 33 IU/mL were considered positive, results < 27 IU/mL were considered negative, and results between ≥ 27 and < 33 IU/mL were considered equivocal [5]. Equivocal results were confirmed by at least two repeated tests and coded as negative. Quality control samples were included on each plate. The results are reported as index values or IU/mL, traceable to the WHO Anti-Toxoplasma Serum, 3rd International Standard Preparation, 1994. Prior to the study, the assay was evaluated against the CDC Toxoplasma immunofluorescence assay and the Sabin–Feldman dye test and demonstrated 100% sensitivity and specificity. Serological test results were useful as presumptive evidence for T. gondii infection [5].

Definition of CVD mortality

Death data were obtained by linking the cohort database to the National Death Index until December 31, 2019. CVD mortality was defined using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision codes I00–I09, I11, I13, I20–I51, and I60–I69 [21].

Covariates

Demographic variables include age, sex, race/ethnicity, body mass index (BMI), educational level, poverty-to-income ratio, smoking status, diabetes, CVD, hypertension, healthy eating index (HEI), physical activity, alcohol intake, cognitive performance, and estimated glomerular filtration rate (eGFR). In our analysis, we treated age as a continuous variable for regression analysis. Race/ethnicity was classified as Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, or Other including multiracial. Education level was divided into three categories: <9 years, 9–13 years, and ≥ 13 years. We also categorized birthplace as U.S.-born or non-U.S.-born. To calculate the income-to-poverty ratio, we divided household income by poverty guidelines based on the number of persons in the household, appropriate year, and state. Smoking status was classified as current smoker, former smoker, or never smoker. We defined hypertension as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, self-reported hypertension subsequently diagnosed by a physician, or elevated blood pressure requiring antihypertensive medication [22]. Hyperlipidemia was defined as triglyceride levels of ≥ 150 mg/dL, cholesterol levels of ≥ 200 mg/dL (total cholesterol), LDL of ≥ 130 mg/dL, or HDL < 40 mg/dL in men and < 50 mg/dL in women, or taking cholesterol-lowering drugs [23]. We also defined diabetes as being diagnosed by a physician as diabetic, taking glucose-lowering medication, having a glycosylated hemoglobin level ≥ 6.5% or a fasting blood glucose level ≥ 126 mg/dL [24]. CVD was defined as heart disease, coronary artery disease, stroke, or congestive heart failure as diagnosed by a physician [25]. Cancer was defined as a diagnosis of cancer or malignancy by a physician. To calculate the HEI, we used the HEI-2015 guidelines, with higher scores indicating a better-quality diet. We classified physical activity levels as active or inactive [21], and we categorized alcohol intake as none, moderate (0.1 to 27.9 g/day for men and 0.1 to 13.9 g/day for women), or heavy (≥ 28 g/day for men and ≥ 14 g/day for women) [22]. Finally, we assessed cognitive function

using three tests: Consortium to Establish a Registry for Alzheimer's Disease (CERAD) word learning and recall modules, the Animal Fluency (AF) test, and the Digit Symbol Substitution Test (DSST). We calculated a composite score by summarizing the z-scores from these tests to represent overall cognitive performance, with higher scores indicating better cognitive performance. We classified participants as having normal or poor cognitive performance based on a threshold set at the 25th percentile [26].

Statistical analysis

Descriptive statistical analyses were conducted on the data collected from all patients. Categorical variables are reported as frequencies and percentages. For normally distributed data, continuous variables are reported as means and standard deviations (SD), or medians and interquartile ranges. The chi-square test, one-way ANOVA, and Kruskal–Wallis test were used for categorical, normally distributed, and non-normally distributed continuous variables, respectively. A Cox proportional hazards model was used to assess the effects of T. gondii infection on CVD mortality. We constructed three multivariate models. Model I was adjusted for age (continuous, years) and sex (male and female). In Model II, the outcomes were further evaluated by adjusting for birthplace (U.S.-born or non-U.S.-born), race/ethnicity (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, or other, including multi-racial), education level (< 9 years, 9–13 years, or ≥ 13 years), poverty-to-income ratio (continuous), BMI (continuous), smoking status (never smoker, former smoker, or current smoker), and HEI (continuous). In Model III, the results were further adjusted for comorbidities (hyperlipidemia, diabetes, hypertension, and CVD), physical activity (inactive or active), alcohol intake (none, moderate, or heavy), and eGFR (continuous). Kaplan–Meier curves were used to assess the impact of T. gondii IgG antibody positivity on CVD mortality in both men and women, with adjustment for all covariates in Model III. The covariates were selected based on established scientific research, traditional risk factors for overall mortality, or observed changes of more than 10% in the effect estimates within the Cox regression model. To prevent overfitting due to multicollinearity among variables, we also calculated the variance inflation factor (VIF) and excluded variables with a VIF of ≥ 5.

For the male population, the analysis was further stratified by age (< 60 or ≥ 60 years), birthplace (U.S.-born or non-U.S.-born), race-ethnicity (non-white or other), BMI (< 25 or ≥ 25 kg/m^2), smoking status (never smoker, former smoker, or current smoker), hyperlipidemia (yes or no), diabetes (yes or no), hypertension (yes or no), CVD (yes or no), physical activity (inactive or active), eGFR(< 60 or ≥ 60 mL/min/1.73 m^2) and alcohol intake (none, moderate, or heavy). We assessed interactions using P-values for the production terms between the T. gondii antibody (negative or positive) and stratified factors.

We conduct a range of sensitivity analyses to strengthen the robustness of our findings. First, we utilized multiple imputations based on five replications of the Markov chain Monte Carlo method to calculate missing data. Second, to mitigate the potential for reverse causality bias, we excluded participants who died within two years of follow-up. Third, we excluded 26 HIV-positive patients who were at a high risk of death due to T. gondii infection and repeated the main analysis. Fourth, cognitive function was an important confounding factor in this study, given that patients with impaired cognitive function have a higher rate of cardiovascular events [27], and T. gondii infection has also been shown to be associated with reduced cognitive function [28]. However, cognitive function was only tested in NHANES for those aged ≥ 60 years or older from 2011 to 2014, which would result in a large amount of missing data if included as a covariate in the model. Therefore, we present a full model with additional adjusted covariates for the sensitivity analysis. Finally, we ran a competing risk analysis, in which deaths due to non-CVD causes were analyzed as the competing cause of death to strengthen our conclusions.

All statistical analyses were conducted using R version 4.1.3, and Free Statistics software version 1.8. Statistical significance was defined as a two-tailed P-value of < 0.05.

Results

Study participants and baseline characteristics

As shown in Table 1, in both male and female participants, T. gondii-seropositive patients tended to be older, not non-Hispanic white, non-U.S. born, had lower education level, and poverty income ratio, combined with hypertension, hyperlipidemia, CVD, diabetes, and CKD, higher HEI, physical inactivity, and low-frequency alcohol use. In addition, T. gondii-seropositive male patients had lower cognitive function and higher rates of CVD mortality. In the female population, T. gondii-seropositive patients had a higher BMI than seronegative patients.

Effects of T. gondii-seropositivity on CVD mortality

In Model III (fully adjusted model), compared with the T. gondii-seronegative group, the HR of the T. gondii-seropositivity group, in all participants, male and female were 1.17 (95% CI: 0.91–1.51), 1.40 (95% CI: 1.02–1.93), and 0.87 (95% CI: 0.57–1.34) after adjusting for all covariates in Model III (Table 2). In Fig. 2, Kaplan-Meier analysis

revealed a significant difference in survival time between two groups of male individuals.

Stratified and sensitivity analyses

Among the male participants, T. gondii-seropositivity were positively associated with CVD mortality. In the stratified analysis there was no evidence that the relationship between T. gondii-seropositivity and CVD mortality changed by age (< 60 or ≥ 60 years), birth place (U.S. born, non-U.S. born), race-ethnicity (non-white or other), poverty income ratio(< 1.0, 1.0–3.0, ≥ 3.0), BMI (< 25, ≥ 25 kg/m^2), smoking status (never smoker, former smoker, and current smoker), hyperlipidemia (yes or no), diabetes (yes or no), hypertension (yes or no), CVD (yes or no), physical activity (inactive, active), and alcohol intake (none, moderate, heavy), or eGFR(< 60, ≥ 60 mL/min/1.73 m^2) (Fig. 3).

A multiple imputation sensitivity analysis did not significantly alter the results. (eTable 1). The results were generally robust in the sensitivity analyses when excluding participants who died within 2 years of follow-up (eTable 2) and when excluding participants with positive serum HIV antibodies (eTable 3), further adjusting for cognitive performance (eTable 4). In a competing risk analysis in the male population, the positive association of T. gondii-seropositivity with CVD death did not change. (eTable 5).

Discussion

In this study, we analyzed data from the NHANES from 2009 to 2014 and identified a new association between T. gondii infection and CVD mortality risk. Specifically, our results indicate that men with T. gondii infection are at an increased risk of CVD mortality, whereas no significant association was observed among women. These findings were robust after adjusting for potential confounding factors and were supported by additional sensitivity analyses, suggesting that T. gondii infection may be an independent risk factor for CVD mortality in men.

T. gondii exhibits tropism for multiple organs, including the myocardium [8]. While the central nervous system is predominantly affected in immunocompromised individuals [4], cardiac involvement is often underdiagnosed due to its asymptomatic nature or obscuration by neurological manifestations [29]. Nevertheless, several epidemiological studies have demonstrated an association between T. gondii seropositivity and cardiovascular disease (CVD). Cross-sectional analyses in Iranian and Mexican populations revealed significantly higher T. gondii seroprevalence among patients with myocardial infarction and other CVDs compared to healthy controls [30–32]. However, the cross-sectional design of these studies precludes the establishment of causality or the assessment of cumulative risk between T. gondii infection and CVD.

In our study, we found that the prevalence of CVD was significantly higher in both men and women with T. gondii infection than in those without infection, which is consistent with previous literature. Furthermore, our analysis of a median follow-up of eight years revealed that T. gondii infection was an independent risk factor for CVD mortality in men but not in women, even after adjusting for important covariates, such as cardiovascular history. This finding suggests that T. gondii infection may be a significant contributor to cardiac risk, particularly in men. T. gondii infection may contribute to cardiovascular risk through multiple mechanisms. In toxoplasmosis, the parasite invades cardiomyocytes through actin-based motility, establishing intracellular vacuoles derived from the plasma membrane [29]. The extent of cardiac tissue damage depends on the intensity of inflammatory reactions and the intramyocytic presence of T. gondii tachyzoites [10]. This invasion can lead to myocardial injury, potentially causing atrial and peripheral arrhythmias, heart failure, atrioventricular block, and even sudden death [20, 29]. Furthermore, T. gondii infection has been associated with elevated levels of chronic inflammation markers such as C-reactive protein (CRP) and biomarkers of vascular injury, including systolic blood pressure, low-density lipoprotein cholesterol, triglycerides, and gamma-glutamyltransferase [17, 33]. These factors may exacerbate existing atherosclerotic lesions and increase the risk of plaque instability and rupture, potentially heightening the risk of myocardial infarction in patients with existing coronary artery occlusion or atherosclerosis. Although there is no direct evidence of T. gondii physically adhering to vascular walls or fat, the parasite's surface and secreted antigens may interact with host cell receptors. These interactions could potentially increase the production of nitric oxide (NO) and reactive oxygen species (ROS), leading to enhanced vascular constriction and oxidative stress in tissues [18], thereby promoting lipid accumulation, foam cell formation, and the release of inflammatory factors, indirectly exacerbating vascular obstruction. In our study, we observed that T. gondii-infected populations had a higher prevalence of CVD-associated chronic diseases, including hypertension, hyperlipidemia, diabetes mellitus, and chronic kidney disease. Notably, even after adjusting for these cardiovascular-related covariates, T. gondii infection remained positively associated with CVD mortality in men, suggesting an independent contribution to CVD mortality. These findings underscore the complex relationship between T. gondii infection and cardiovascular health,

Table 1 Baseline characteristics of the study participants

Variables	Toxoplasma gondii antibody							
	Male				Female			
	Total ($n=5010$)	Negative ($n=4122$)	Positive ($n=888$)	*p*-value	Total ($n=5227$)	Negative ($n=4483$)	Positive ($n=744$)	*p*-value
Age, years, Mean ± SD	46.1 ± 16.5	44.8 ± 16.1	52.5 ± 16.6	< 0.001	46.8 ± 16.7	45.8 ± 16.6	52.5 ± 16.3	< 0.001
Race-ethnicity, n (%)				< 0.001				< 0.001
Non-Hispanic White	2216 (44.2)	1899 (46.1)	317 (35.7)		2285 (43.7)	2026 (45.2)	259 (34.8)	
Non-Hispanic Black	953 (19.0)	784 (19.0)	169 (19.0)		1043 (20.0)	891 (19.9)	152 (20.4)	
Other Hispanic	475 (9.5)	308 (7.5)	167 (18.8)		540 (10.3)	376 (8.4)	164 (22.0)	
Mexican American	779 (15.5)	622 (15.1)	157 (17.7)		762 (14.6)	655 (14.6)	107 (14.4)	
Other Race	587 (11.7)	509 (12.3)	78 (8.8)		597 (11.4)	535 (11.9)	62 (8.3)	
Birth place, n (%)				< 0.001				< 0.001
US born	3557 (71.0)	3095 (75.1)	462 (52.0)		3780 (72.3)	3393 (75.7)	387 (52.0)	
Non-US born	1453 (29.0)	1027 (24.9)	426 (48.0)		1447 (27.7)	1090 (24.3)	357 (48.0)	
Poverty Income Ratio, Mean ± SD	2.6 ± 1.7	2.6 ± 1.7	2.2 ± 1.6	< 0.001	2.4 ± 1.6	2.5 ± 1.7	2.1 ± 1.5	< 0.001
Education Level, n (%)				< 0.001				< 0.001
Low (< 9 years)	456 (9.1)	298 (7.2)	158 (17.8)		438 (8.4)	324 (7.2)	114 (15.3)	
Medium (9–13 years)	1918 (38.3)	1539 (37.3)	379 (42.7)		1775 (34.0)	1467 (32.7)	308 (41.4)	
High (≥ 13 years)	2636 (52.6)	2285 (55.4)	351 (39.5)		3014 (57.7)	2692 (60.0)	322 (43.3)	
BMI, kg/m^2, Mean ± SD	28.8 ± 6.1	28.8 ± 6.1	28.8 ± 6.1	0.916	29.6 ± 7.6	29.4 ± 7.7	30.6 ± 7.2	< 0.001
Smoking status, n (%)				0.148				0.015
Former Smoker	1317 (26.3)	1074 (26.1)	243 (27.4)		910 (17.4)	767 (17.1)	143 (19.2)	
Never Smoker	2448 (48.9)	2040 (49.5)	408 (45.9)		3376 (64.6)	2882 (64.3)	494 (66.4)	
Current Smoker	1245 (24.9)	1008 (24.5)	237 (26.7)		941 (18.0)	834 (18.6)	107 (14.4)	
Hyperlipidemia, n (%)	3483 (69.5)	2822 (68.5)	661 (74.4)	< 0.001	3737 (71.5)	3162 (70.5)	575 (77.3)	< 0.001
Hypertension, n (%)	1915 (38.2)	1529 (37.1)	386 (43.5)	< 0.001	1977 (37.8)	1638 (36.5)	339 (45.6)	< 0.001
Diabetes, n (%)	826 (16.5)	641 (15.6)	185 (20.8)	< 0.001	809 (15.5)	663 (14.8)	146 (19.6)	< 0.001
CVD, n (%)	494 (9.9)	353 (8.6)	141 (15.9)	< 0.001	357 (6.8)	287 (6.4)	70 (9.4)	0.003
Cognitive Performance[a], n (%)				0.005				0.815
Low	226 (29.8)	150 (27.0)	76 (37.6)		179 (22.2)	146 (22.0)	33 (22.9)	
Normal	532 (70.2)	406 (73.0)	126 (62.4)		628 (77.8)	517 (78.0)	111 (77.1)	
HEI, Mean ± SD	50.2 ± 13.5	50.0 ± 13.5	51.3 ± 13.6	0.007	51.9 ± 14.0	51.6 ± 13.9	53.6 ± 14.2	< 0.001
Physical Activity, n (%)				< 0.001				0.001
Inactive	2335 (46.6)	1824 (44.3)	511 (57.5)		2702 (51.7)	2276 (50.8)	426 (57.3)	

Table 1 (continued)

Variables	Toxoplasma gondii antibody							
	Male				Female			
	Total ($n=5010$)	Negative ($n=4122$)	Positive ($n=888$)	*p*-value	Total ($n=5227$)	Negative ($n=4483$)	Positive ($n=744$)	*p*-value
Active	2675 (53.4)	2298 (55.7)	377 (42.5)		2525 (48.3)	2207 (49.2)	318 (42.7)	
Alcohol Intake, n (%)				0.001				< 0.001
None	3416 (68.2)	2772 (67.2)	644 (72.5)		4226 (80.8)	3584 (79.9)	642 (86.3)	
Moderate	406 (8.1)	330 (8.0)	76 (8.6)		416 (8.0)	364 (8.1)	52 (7.0)	
Heavy	1188 (23.7)	1020 (24.7)	168 (18.9)		585 (11.2)	535 (11.9)	50 (6.7)	
eGFR, mL/min/1.73 m^2, Mean ± SD	94.8 ± 21.3	95.8 ± 20.8	89.9 ± 22.9	< 0.001	97.3 ± 23.4	98.0 ± 23.2	93.5 ± 24.2	< 0.001
Follow-up in years, Median (IQR)	8.0 (6.3, 9.6)	7.9 (6.3, 9.6)	8.2 (6.5, 9.6)	0.229	8.0 (6.4, 9.6)	7.9 (6.3, 9.6)	8.2 (6.6, 9.7)	0.027
CVD Mortality, n (%)	177 (3.5)	111 (2.7)	66 (7.4)	< 0.001	135 (2.6)	108 (2.4)	27 (3.6)	0.052

BMI body mass index, *CVD* cardiovascular disease, *eGFR* estimated glomerular filtration rate, *HEI* healthy eating index

[a] cognitive function was only tested in NHANES for those aged ≥ 60 years or older from 2011–2014

highlighting the need for further research to elucidate the precise mechanisms and potential causal relationships.

The second important finding of our study was the sex difference in CVD mortality associated with T. gondii infection. However, the underlying mechanisms remain unclear. One possible explanation is the sex-specific behavioral and neurological effects of T. gondii infection in humans. Literature suggests that T. gondii infection causes alterations in dopamine levels, which can affect human behavior [34]. Behavioral changes induced by potential toxoplasmosis also vary according to the host sex [35]. In a U.S. population-based survey, sex differences in tobacco use were observed among individuals with T. gondii infections [36]. Specifically, seropositive men were more likely to self-report smoking than seronegative men, whereas seropositive women were less likely to self-report smoking than seronegative women. In addition, seropositive men were more likely to report lifetime alcohol consumption than seronegative men. In the present study, we did not observe a difference in the proportion of men who self-reported smoking between the T. gondii -positive and-negative populations. However, similar to the findings in the literature, we found that the proportion of T. gondii-positive women who self-reported smoking was lower than that of T. gondii-negative women. This observation suggests that T. gondii may contribute to sex differences in the risk of CVD mortality by modifying smoking behavior. Additionally, alcohol consumption is a strong predisposing factor for T. gondii invasion and the transfer of host cells

Table 2 Multivariable Cox regression analysis Toxoplasma Gondii seropositivity and cardiovascular mortality

Variable	Total *n*	Deaths *n*(%)	Non-adjusted Model HR (95% CI)	*p* Value	Model I HR (95% CI)	*p* Value	Model II HR (95% CI)	*p* Value	Model III HR (95% CI)	*p* Value
All participants										
Negative	8605	219 (2.5)	Ref.		Ref.		Ref.		Ref.	
Positive	1632	93 (5.7)	2.21 (1.74 ~ 2.82)	< 0.001	1.24 (0.97 ~ 1.58)	0.088	1.19 (0.92 ~ 1.52)	0.183	1.17 (0.91 ~ 1.51)	0.214
Male										
Negative	4122	111 (2.7)	Ref.		Ref.		Ref.		Ref.	
Positive	888	66 (7.4)	2.74 (2.02 ~ 3.72)	< 0.001	1.52 (1.11 ~ 2.06)	0.008	1.45 (1.06 ~ 2.00)	0.021	1.40 (1.02 ~ 1.93)	0.039
Female										
Negative	4483	108 (2.4)	Ref.		Ref.		Ref.		Ref.	
Positive	744	27 (3.6)	1.48 (0.97 ~ 2.25)	0.070	0.90 (0.59 ~ 1.37)	0.610	0.86 (0.56 ~ 1.33)	0.506	0.87 (0.57 ~ 1.34)	0.529

Model I: adjusted for age + sex; Model II: adjusted for Model I+birth place + race + education + poverty income ratio + BMI + smoking status + HEI; Model III: adjusted for Model II+diabetes + hypertension + hyperlipidemia + CVD + physical activity + alcohol intake + eGFR

BMI body mass index, *CVD* cardiovascular disease, *eGFR* estimated glomerular filtration rate, *HEI* healthy eating index

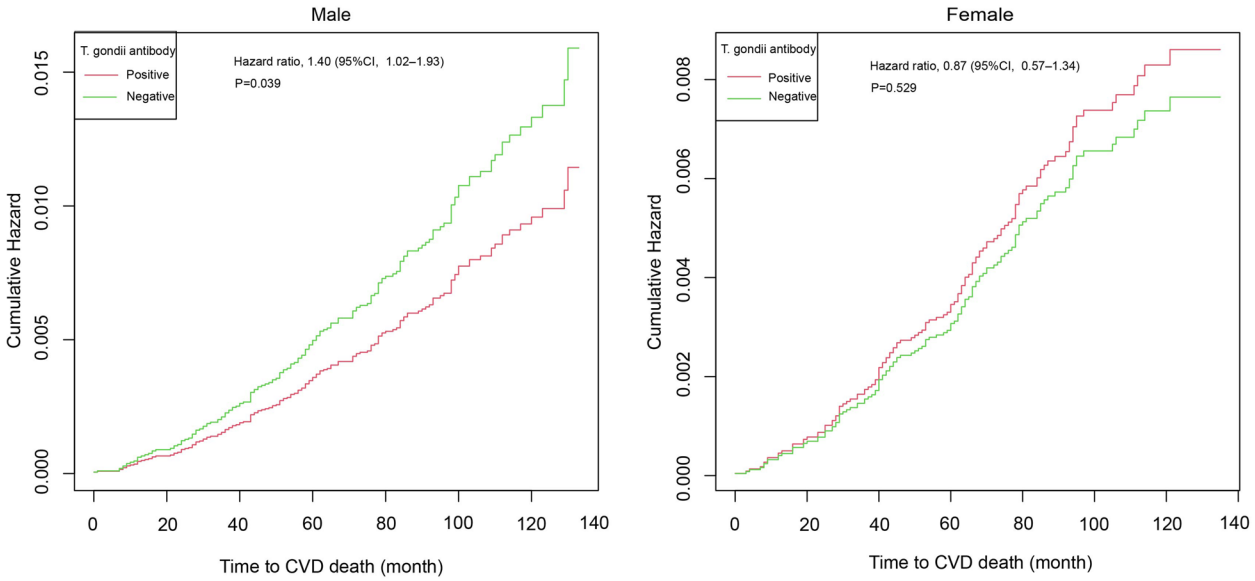


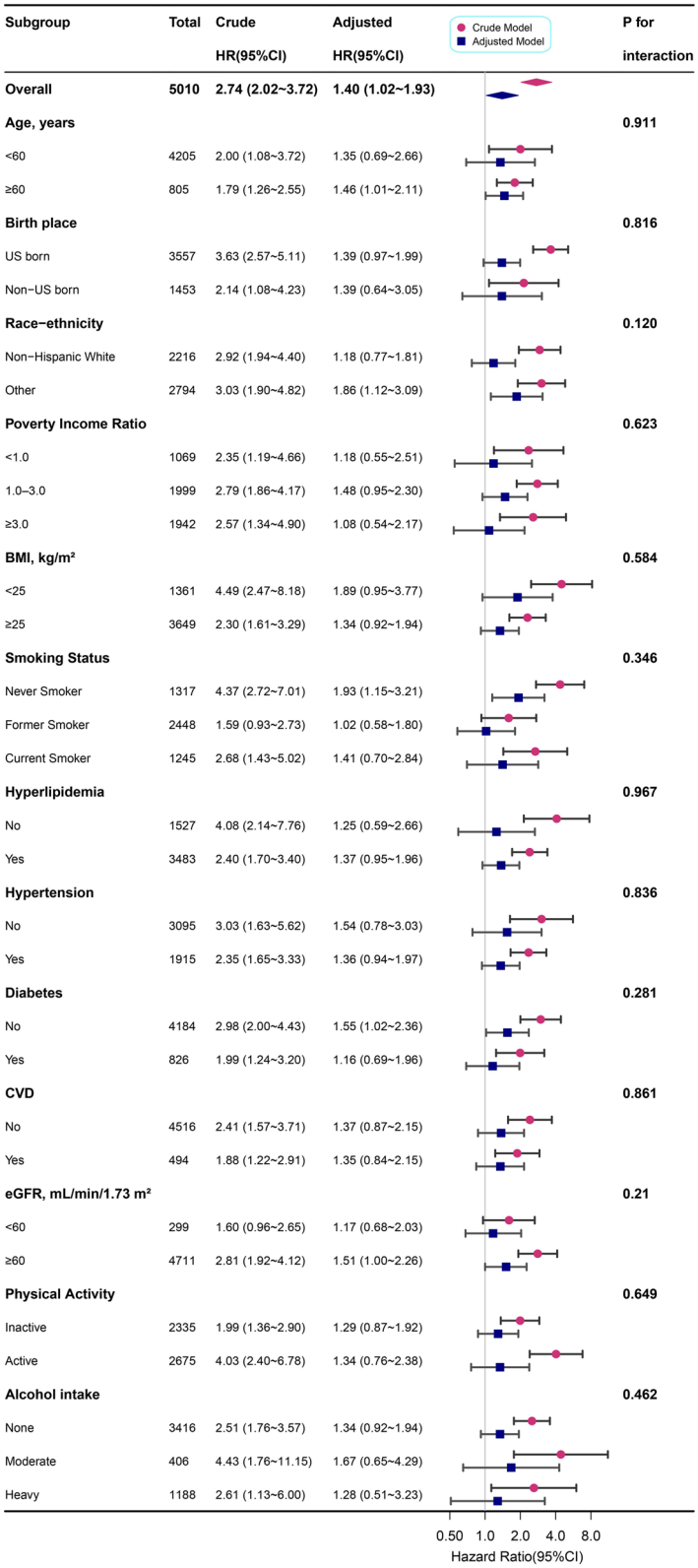
Fig. 2 Sex-specific Kaplan-Meier Analysis: Toxoplasma gondii Seropositive vs. Seronegative Groups. Kaplan-Meier estimates were obtained from the multivariable model adjusted for age, race, birth place, education, BMI, poverty income ratio, smoking status, diabetes, hypertension, CVD, hyperlipidemia, physical activity, alcohol intake, eGFR, HEI. **Abbreviations**: BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HEI, healthy eating index

[37]. Alcohol consumption, a prerequisite for the development of CVD, may make the host more vulnerable to T. gondii attacks. Men tend to have a higher frequency of alcohol consumption than women, which further increases the risk of CVD after infection. Another possible explanation for the sex differences in CVD mortality associated with T. gondii infection is the sex-specific neurological effects. An in vitro assay of the molecular changes induced by persistent T. gondii infection in mice revealed significant sex-dependent transcription of genes in the frontal cortex [38]. In the general population, males showed a lower sense of rules and greater enjoyment after T. gondii infection, whereas females showed promiscuity and a greater sense of responsibility [35]. The effect of T. gondii infection on cognitive impairment in our study also showed sex differences, with a higher proportion of men with cognitive impairment in the T. gondii-seropositive population than in the T. gondii-seronegative population. However, no such differences were observed in the women. Since cognitive impairment has been shown to be a risk factor for CVD [27], this may contribute to sex differences in CVD by T. gondii infection. Although we attempted to explain the sex differences in the association between T. gondii infection and CVD through the abovementioned factors, it is important to note that the sex differences in smoking status, drinking status, and cognitive function observed between the T. gondii -seropositive and T. gondii -seronegative groups were based on baseline survey data and did not establish causality between these behavioral and cognitive changes and T. gondii infection. This partially explains why the positive association between T. gondii seropositivity and CVD mortality persisted in the male population even after further adjustment for smoking status, alcohol intake, and cognitive performance. However, further prospective studies are required to confirm these findings.

Given the widespread prevalence of T. gondii infection globally, these findings suggest that T. gondii seropositivity could be considered as an additional risk factor in cardiovascular health assessments. This may necessitate the development of targeted screening and preventive strategies, especially for men with T. gondii infection, to mitigate their elevated risk of CVD mortality. Furthermore, these results underscore the importance of public health measures aimed at reducing T. gondii transmission,

(See figure on next page.)

Fig. 3 Associations between Toxoplasma gondii seropositivity and cardiovascular mortality in various subgroups among male patients. Toxoplasma gondii seronegativity was used as the reference level in all the models. Age, race, birth place, education, BMI, poverty income ratio, smoking status, diabetes, hypertension, CVD, hyperlipidemia, physical activity, alcohol intake, eGFR, HEI were adjusted. The strata variable was not included when stratifying by itself. Abbreviations: BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HEI, healthy eating index



Subgroup	Total	Crude HR(95%CI)	Adjusted HR(95%CI)	P for interaction
Overall	**5010**	**2.74 (2.02~3.72)**	**1.40 (1.02~1.93)**	
Age, years				**0.911**
<60	4205	2.00 (1.08~3.72)	1.35 (0.69~2.66)	
≥60	805	1.79 (1.26~2.55)	1.46 (1.01~2.11)	
Birth place				**0.816**
US born	3557	3.63 (2.57~5.11)	1.39 (0.97~1.99)	
Non−US born	1453	2.14 (1.08~4.23)	1.39 (0.64~3.05)	
Race−ethnicity				**0.120**
Non−Hispanic White	2216	2.92 (1.94~4.40)	1.18 (0.77~1.81)	
Other	2794	3.03 (1.90~4.82)	1.86 (1.12~3.09)	
Poverty Income Ratio				**0.623**
<1.0	1069	2.35 (1.19~4.66)	1.18 (0.55~2.51)	
1.0–3.0	1999	2.79 (1.86~4.17)	1.48 (0.95~2.30)	
≥3.0	1942	2.57 (1.34~4.90)	1.08 (0.54~2.17)	
BMI, kg/m²				**0.584**
<25	1361	4.49 (2.47~8.18)	1.89 (0.95~3.77)	
≥25	3649	2.30 (1.61~3.29)	1.34 (0.92~1.94)	
Smoking Status				**0.346**
Never Smoker	1317	4.37 (2.72~7.01)	1.93 (1.15~3.21)	
Former Smoker	2448	1.59 (0.93~2.73)	1.02 (0.58~1.80)	
Current Smoker	1245	2.68 (1.43~5.02)	1.41 (0.70~2.84)	
Hyperlipidemia				**0.967**
No	1527	4.08 (2.14~7.76)	1.25 (0.59~2.66)	
Yes	3483	2.40 (1.70~3.40)	1.37 (0.95~1.96)	
Hypertension				**0.836**
No	3095	3.03 (1.63~5.62)	1.54 (0.78~3.03)	
Yes	1915	2.35 (1.65~3.33)	1.36 (0.94~1.97)	
Diabetes				**0.281**
No	4184	2.98 (2.00~4.43)	1.55 (1.02~2.36)	
Yes	826	1.99 (1.24~3.20)	1.16 (0.69~1.96)	
CVD				**0.861**
No	4516	2.41 (1.57~3.71)	1.37 (0.87~2.15)	
Yes	494	1.88 (1.22~2.91)	1.35 (0.84~2.15)	
eGFR, mL/min/1.73 m²				**0.21**
<60	299	1.60 (0.96~2.65)	1.17 (0.68~2.03)	
≥60	4711	2.81 (1.92~4.12)	1.51 (1.00~2.26)	
Physical Activity				**0.649**
Inactive	2335	1.99 (1.36~2.90)	1.29 (0.87~1.92)	
Active	2675	4.03 (2.40~6.78)	1.34 (0.76~2.38)	
Alcohol intake				**0.462**
None	3416	2.51 (1.76~3.57)	1.34 (0.92~1.94)	
Moderate	406	4.43 (1.76~11.15)	1.67 (0.65~4.29)	
Heavy	1188	2.61 (1.13~6.00)	1.28 (0.51~3.23)	

Fig. 3 (See legend on previous page.)

which could potentially have a beneficial impact on cardiovascular health outcomes at the population level.

Our study has several strengths. This is the first investigation of the association between T. gondii infection and CVD mortality. Second, we used a prospective cohort design with a large sample size from a nationally representative sample. Moreover, we obtained a relatively long follow-up period of up to eight years and a low incidence of mismatched records in the NHANES-linked mortality file. Additionally, because the NHANES collects comprehensive data, we were able to control for a wide range of potential confounding effects of demographic, socioeconomic, lifestyle, and dietary factors. Despite the valuable insights gained from this study, several limitations must be considered when interpreting the findings. First, positive T. gondii IgG serology results only indicated a previous infection, and we were unable to determine the exact timing of the T. gondii infection or its cumulative effect on the risk of CVD mortality. Second, although we adjusted for many known risk factors, the observational nature of our study means that residual or unmeasured confounders could not be completely excluded. Third, the self-reported diagnoses of many chronic diseases may be subject to recall bias. Furthermore, although the NHANES dataset is designed to be representative of the U.S. population, our findings may not be fully generalizable to other populations or geographic regions. Differences in T. gondii infection rates, distribution of cardiovascular risk factors, and healthcare systems across different areas may affect the universal applicability of our results. Future studies should validate these findings in diverse populations and geographical contexts to address these limitations and further elucidate the relationship between T. gondii infection and CVD mortality.

Conclusions

In conclusion, our study demonstrated that T. gondii infection is associated with increased CVD mortality, particularly in men. The mechanisms underlying this association are not fully understood; however, our findings suggest that T. gondii infection may affect CVD through multiple pathways, including mechanical damage to the heart, inflammatory responses, and sex-specific behavioral and neurological effects. Further research is needed to elucidate the mechanisms underlying the association between T. gondii infection and CVD mortality, and to identify potential interventions to reduce this risk.

Abbreviations

NHANES	National Health and Nutrition Examination Survey
NCHS	National Center for Health Statistics
CDC	Centers for Disease Control
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
AF	Animal Fluency
DSST	Digit Symbol Substitution Test
BMI	body mass index
CVD	cardiovascular disease
eGFR	estimated glomerular filtration rate
HEI	healthy eating index

Supplementary Information

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Supplementary Material 1

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Authors' contributions

HL, YX and WQ designed the study and wrote the article. LZ and ZX designed the study and analyzed the data. ZC, HL were involved in manuscript preparation and data collection. All authors read and approved the final manuscript.

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Availability of data and materials

Publicly available datasets were analyzed in this study. The raw data used in the article are available on National Health and Nutrition Examination Survey website (https://www.cdc.gov/nchs/nhanes/index.htm).

Declarations

Ethics approval and consent to participate

The study received approval from the Ethics Committee of The Second Affiliated Hospital of Gannan Medical University (EFRJ20230214001).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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