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Original article

Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in a representative Australian human population: The *Busselton* health study

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ABSTRACT

Introduction: Despite being identified as one of the top neglected parasitic infections, *Toxoplasma gondii* has received little recognition in Australia with no previously published prevalence data from the general human population. The objectives of the current study were to: determine the prevalence of evidence of exposure to *T. gondii* infection in an Australian community dwelling population, and: identify associated risk factors.

Methods: Sera from 75 males and 75 age-matched females living in Busselton, Western Australia were tested for the presence of anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunosorbent assay. Survey derived data were also analysed to evaluate risk factors.

Results: IgG and IgM antibodies were detected in 99 (66.0%) and 15 (10.0%) of subjects, respectively. IgG seroprevalence increased from 44.4% (95% confidence interval (95% CI): 18.9–73.3%) in the 18–34 year-old age group to 81.0% (95% CI: 60.0–92.3%) in the 75–84 age group. The observed IgG seroprevalence increased at a rate of 0.8% with each year of age. No risk factors were identified.

Conclusions: The first study of its kind in Australia found *T. gondii* infection to be highly prevalent. *Toxoplasma gondii* infection has been neglected in Australian notifiable disease programs therefore Australian public health authorities should focus on improving education to raise awareness and commence longitudinal epidemiological data collection to supplement public health models targeting *T. gondii* transmission control.

1. Introduction

First described in 1908 by Nicolle and Manceaux,[1] the obligate intracellular parasite *T. gondii* infects approximately one third of the world's population and is considered one of the most successful human parasites.[2–5] The Centers for Disease Control has prioritised *T. gondii* as one of the top "Five Neglected Parasitic Infections" due to the severity of illness, high incidence, and potential for prevention.[6] Humans acquire *T. gondii* infection by the ingestion of food, water, or soil contaminated by oocysts from the definitive hosts, cats (family, *Felidae*; genera, *Felis* and *Lynx*). *Toxoplasma gondii* is also transmitted in people vertically via placenta and horizontally via blood transfusion.[3,7] Capable of infecting all warm-blooded animals, *T. gondii* infection is one of considerable public health impact. The global prevalence rates of this parasite are remarkable, with figures ranging from 15 to 85% depending on social habits, climate conditions, hygienic standards, and geographical regions.[10] Although *T. gondii* has a worldwide distribution and possibly the widest host range of any parasite, there is only one species (*T. gondii*) in the genus Toxoplasma and the cat family are the only definitive hosts in which *T. gondii* sexual development is known to occur.[8]

Infection starts with an acute phase followed by a chronic phase and this parasite has three infectious stages: sporozoites, tachyzoites and bradyzoites.[9] Due to their active multiplication, tachyzoites cause extensive tissue damage and may disseminate to different tissues of the host. *Toxoplasma gondii* tachyzoites infects circulating white blood cells and can use them as a "Trojan Horse" to gain access through protective tissues to organs such as the brain, a compartment where entry of immune cells is restricted. The tachyzoites eventually undergo conversion and become slowly multiplying bradyzoites within tissue cysts. In instances where the tissue cysts become activated, the bradyzoites can undergo further conversion and become tachyzoites.[7,10] All parasitic stages can infect feline enterocytes leading to oocyst production, which restarts the life cycle of *T. gondii*.[8]

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It should be noted that the term "toxoplasmosis" should be reserved to describe cases where pathological and/or clinical symptoms of the disease caused by *T. gondii* are active while the term "*T. gondii* infection" should be used to describe asymptomatic infection or evidence of the persistence of the parasite in the host (latent or chronic). Infection can present with various nonspecific signs and symptoms, but most have been previously described as similar to flulike indicators.[7] In immunocompetent individuals, it is thought that the disease is asymptomatic and the individual recovers without treatment because of an efficient immune system which limits the spread of the rapidly multiplying tachyzoites.[7] Consequently, people are not routinely screened for evidence of *T. gondii* infection unless they are clinically immunocompromised or pregnant, in which cases infection may lead to serious complications which can be lethal.[10] Acute infection during pregnancy can cause damage to the developing fetus.[10] In all circumstances, specific antibodies to antigens of this parasite remain detectable in the serum throughout the life of the host, with dormant cysts being formed in various anatomical sites, including the central nervous system, often establishing latent toxoplasmosis.[4] *Toxoplasma gondii* can infect and replicate in any nucleated host cells, leading to the production of various inflammatory biomarkers via the acute inflammatory response and antigen-specific adaptive immunity, in parallel to seroconversion.[4] This facilitates a state of chronic inflammation at various anatomical sites in the host.[7] Consequently, chronic *T. gondii* infection has been linked to several autoimmune disorders including thyroid disease, systemic sclerosis, rheumatoid arthritis, and inflammatory bowel syndrome.[11] Several reports have demonstrated a positive correlation between *T. gondii* infection and numerous neurological disorders and cancers.[7,8,12]

Despite the fact that *T. gondii* is distributed worldwide, there is very limited information on the prevalence, incidence rate, epidemiology, and risk factors specific to the general Australian human population-because *T. gondii* infection is not a notifiable disease in Australia and most infections are asymptomatic. However, using *T. gondii* incidence data from overseas foodborne illnesses surveys, Hall and Kirk[13] calculated a conservative incidence of up to 7,150 new symptomatic cases of *T. gondii* infection in Australia each year. In addition, a few publications have reported prevalence rates amongst pregnant woman throughout the country: 35% in Western Australia,[14] 23% in Melbourne,[15] 23% in South Australia,[16] and 26% in Queensland.[16]

The objectives of this study were to: 1) determine the seroprevalence of *T. gondii* infection in a representative Australian population as no data exist to date; and 2) identify risk factors for *T. gondii* infection. We undertook the first age- and gender-matched study in Australia by utilising sera and cross-sectional clinical data (respiratory and chest conditions; various disease states; anthropometric measurements; and biochemical and haematological laboratory test results) collected from a community-dwelling cohort of adults attending the 2005–2007 Busselton Health Survey (BHS) in Western Australia.

2. Materials and methods

The present study describes serology on blood samples and data collected from adult residents of the Western Australian town of *Busselton*, a centre for farming, vineyards, timber, and mineral sands industries. The cross-sectional general population BHS was conducted between 2005 and 2007 with participants recruited from the compulsory electoral role. This survey was conducted by the Busselton Population Medical Research Institute (BPMRI), a prominent biobank. Details on recruitment and study protocols from this survey are described in Musk et al.[17] Ethical approval was obtained from the Edith Cowan University Human Research Ethics Committee (Project Number 16090).

2.1. Study design and criteria for selection of participants

Through an age- and gender-matched cross-sectional study, the seroprevalence of *T. gondii* was measured in 150 subjects. Sera were analysed for the presence of IgG and IgM antibodies against *T. gondii* using commercially available qualitative ELISA methods (Demeditec Diagnostics GmbH, Germany). These kits have claimed clinical sensitivities and specificities of 99% and 98%, respectively, for the IgG assay; 99% and 100%, respectively, for the IgM assay. Survey-derived data including respiratory and chest conditions; various disease states; together with anthropometric measurements; details of pet ownership; and biochemical and haematological laboratory test results were investigated for possible significant risk factor association. Participants were recruited by reference to the following criteria: 1) do not have a documented medical diagnosis of diabetes, 2) not taking any glucose-lowering medications, and 3) have fasting and 2-h glucose values below the diagnostic thresholds for diabetes. In addition, subjects were screened for history of treatment with psychoactive medication and were excluded. These criteria were selected because of the previously reported and established associations between positive *T. gondii* serology, diabetes, and numerous forms of serious mental illnesses.[8,11,18,19]

2.2. Sample and data collection

This study utilised 20 μL of serum aliquoted from banked samples (stored at −80 °C) that had been previously collected by the BPMRI from volunteers aged 18–80 years during the 2005–2007 BHS. Each participant was required to complete a standard self-administered questionnaire that obtained information on respiratory and chest conditions; various disease states; and pet ownership. Each participant had anthropometric and body composition measurements taken in addition to blood samples collected from the cubital fossa in 10 mL Red top-clot, 2 mL Purple top-EDTA, and 2 mL Grey Top -FlOx Vacutainer tubes (BD Biosciences, Franklin Lakes, New Jersey, USA) for laboratory biochemical and haematological analyses which were conducted by PathWest Laboratory Medicine- QEII Medical Centre, Nedlands, Western Australia. Serum for the present study was aliquoted and stored at −80 °C until used.

2.3. Sample integrity and laboratory analysis

Lipemic, haemolysed, icteric, or turbid (bacterially contaminated) samples were excluded as they may cause false positive or false negative results. Test samples were diluted 1:101 with ready-to-use sample diluent (5 μL serum + 500 μL sample diluent). The total volume of serum required from each subject to perform the IgG and IgM ELISAs was 20 μL (5 μL for IgG ELISA, 5 μL for IgM ELISA, and 10 μL for repeat/s in the event of equivocal result/s). The sera were tested for anti-*T. gondii* IgG and anti-*T. gondii* IgM antibodies using the Demeditec Diagnostics DETOX01 (IgG ELISA) and DETOX03 (IgM ELISA) kits according to the manufacturers' instructions. Briefly, these assays have been designed for the qualitative evaluation of specific IgG and IgM antibodies against Toxoplasma in serum. The analysis was performed double-blind to avoid result bias. Samples from the female and male groups were randomly sorted/selected, and the analyst performing the analysis was not aware of the source of samples. Then,100 μL of the diluted (1:101) sample and the ready-to-use calibrators (IgG [IU/mL]; A, 0; B, 10; C, 40; D, 100; E, 250; IgM [U/mL]; A, 1; B, 10; C, 30; D, 120) were pipetted into each test well (coated with *T. gondii* strain RH antigens, isolated from infected mice, common to both the IgG and IgM assays) leaving one well empty for the substrate blank. The plate was covered and incubated for 60 min at room temperature. The wells were then washed three times with 300 μL of diluted washing solution using a Bio-Plex Pro II Microplate Wash Station (Bio-Rad Laboratories, Berkeley, California). Subsequently, 100 μL of ready-to-use conjugate

was added into each well except the substrate blank well. The plate was covered and incubated at room temperature for 30 min. This was followed by another washing procedure as outlined above, after which 100 µL of the ready-to-use substrate was pipetted into each well including the substrate blank well. A final incubation phase for 20 min at room temperature in the dark was performed before terminating the substrate reaction with the addition of 100 µL of the ready-to-use stop solution into each well. The plate was then mixed and the wiped in preparation for reading. This was performed using a FLUOstar Omega microplate reader (BMG Labtech, Offenburg, Germany) at an absorption of 450 nm. A standard curve was generated by plotting the mean absorbance at 450 nm for each standard concentration (x axis) against the target antibody concentration (y axis). This was used to determine the qualitative concentration of target antibody in each sample. By the criteria of the assays used, values higher than the cut-offs (IgG, 10 IU/mL; IgM, 10 U/mL) were considered positive, values less than the cut-offs were considered negative, while values falling within a grayzone of +/-20% of the cut-off values were considered equivocal and retested once.

2.4. Statistical analysis

Basic descriptive statistics were calculated for the seropositive and seronegative groups linking gender and age variables. Seroprevalence of *T. gondii* along with the odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated. The Chi-squared test was used to evaluate the seroprevalence values between positive and negative subjects with respect to demographic categorical variables (health and diseases states, pet ownership) to examine possible significant risk factor associations. For continuous quantitative variables (anthropometric and body composition measurements, laboratory results), the normality of the data distribution was assessed using the Shapiro-Wilk test in addition to Q-Q plots and skewness and kurtosis values. As the laboratory and anthropometric were normally distributed, the Student's t-test was used to compare selected parameters between the study groups. Probability values were calculated on the basis of two-tailed tests. For all analyses, a p value less than 0.05 was considered statistically significant. Data was analysed using IBM® SPSS® Statistics version 25.0 software (SPSS Inc., Armonk, New York, USA).

3. Results

In this study, the 150 selected subjects were matched for age, gender, and age group distribution. The mean age was 58.8 ± 15.8 years. Most of patients were in the 55–64 (n = 32) and 65–74 year-old (n = 32) age groups; followed by the 45–54 year-old (n = 31) age group; then the 35–44 (n = 21) and 75–84 year-old (n = 21) age groups. The basic descriptive and biometric statistics are summarised in Table 1.

Table 1
Basic descriptive and biometric statistics for the study group (n = 150) at baseline including age group stratification. BHS, Western Australia.

Parameter	Overall	Males	Females
Number of subjects	150	75	75
Average age (± SD)	58.8 ± 15.8	63.5 ± 14.5	54.0 ± 15.8
Height (cm ± SD)	168.4 ± 9.3	173.9 ± 7.7	163.0 ± 7.5
Weight (Kg ± SD)	77.2 ± 15.6	85.8 ± 13.8	68.6 ± 12.2
Waist (cm ± SD)	91.3 ± 13.2	99.5 ± 10.2	83.3 ± 10.7
BMI (± SD)	27.1 ± 4.5	28.4 ± 3.8	25.9 ± 4.9
Cat owners	20.0%	16.0%	24.0%
Dog owners	37.3%	40.0%	34.7%
Other pet owners	22.7%	21.3%	24.0%

Average age is recorded in years; cm, centimeter; Kg, kilogram; SD, standard deviation; BMI, body mass index.

Table 2
Stratified seroprevalence of IgG and IgM antibodies against *T. gondii* detected by ELISA in adults aged 18 to 84+ years by gender and age group. BHS, Western Australia.

	Toxoplasma IgG/IgM serology					
	POS (n)	NEG (n)	Prevalence	OR	95% CI	p-value
Gender (IgG)						
Female	46	29	61.3%	ref	Ref	ref
Male	53	22	70.7%	1.52	0.77–3.00	0.228
Total	99	51	66.0%	–	–	–
Gender (IgM)						
Female	13	62	17.3%	ref	Ref	ref
Male	2	73	2.7%	0.13	0.03–0.60	0.003
Total	15	135	10.0%	–	–	–
Age Group (IgG)						
18–34	4	5	44.4%	ref	ref	ref
35–44	9	12	42.9%	0.94	0.19–4.52	0.936
45–54	24	7	77.4%	4.29	0.90–20.42	0.057
55–64	20	12	62.5%	2.08	0.47–9.31	0.331
65–74	22	10	68.8%	2.75	0.61–12.48	0.181
75–84	17	4	81.0%	5.31	0.96–29.30	0.046
> 84	3	1	75.0%	3.75	0.27–51.38	0.308
Total	99	51	66.0%	–	–	–

n, number of subjects; POS, number of subjects in which anti-IgG antibodies were detected; NEG, number of subjects in which anti-IgG antibodies were not detected; OR, odds ratio; 95% CI, 95% confidence interval; age is recorded in years.

3.1. Seroprevalence of T. gondii infection and interaction with gender

From the 150 subjects tested during the study period, 99 (66.0%) were IgG seropositive and 15 (10.0%) were IgM seropositive. Female subjects had an overall lower IgG seroprevalence than their male counterparts, 61.3% and 70.7%, respectively (p = 0.228). However, the opposite was seen with IgM seroprevalence with the difference being significant, 17.3% and 2.7%, respectively (p = 0.003). Univariate analysis identified male gender as a possible risk factor for the presence of IgG antibodies (odds ratio (OR): 1.52; 95% CI: 0.77–3.00, p = 0.228) and female gender for the presence of IgM antibodies (OR: 0.13; 95% CI: 0.0–0.60, p = 0.003) [Table 2].

3.2. Seroprevalence of T. gondii infection and interaction with age

IgG seroprevalence increased from 44.4% (95% CI: 18.9–73.3%) in the 18–34 year-old age group to 81.0% (95% CI: 60.0–92.3%) in the 75–84 year-old age group. Therefore, the observed IgG seroprevalence increased at a rate of 0.8% with each year of age (excluding the 18–34 and > 84 year-old age groups due to low numbers of participants and gender skew). IgM was excluded from further analysis due to the low numbers of seropositive subjects. A relationship was observed between age and *T. gondii* IgG seropositivity as higher IgG seroprevalences were observed with increasing age. IgG seropositivity increased rapidly in all age groups remaining at over 60% in the 45 year-old and above age groups [Table 2].

3.3. Toxoplasma gondii seroprevalence and associated demographic factors

Regarding risk factors associated with *T. gondii* infection, univariate analysis showed that the various respiratory and disease conditions (asthma, arthritis, bronchitis, cancer, eczema, food allergies, hay fever, other chest conditions, pleurisy, pneumonia, sinusitis), pet ownership, and anthropometric measurements were not significantly associated with *T. gondii* infection in IgG-seropositive subjects. However, seropositive were more likely than the controls to own a dog (OR = 1.54, p = 0.343) while the seronegative subjects reported higher ownership of pets other than cats or dogs (OR = 0.57, p = 0.157) [Table 3].

Table 3
Univariate analysis of the variables associated with the seroprevalence of anti-*Toxoplasma gondii* IgG antibodies among subjects. BHS, Western Australia.

	Toxoplasma gondii IgG serology					
	POS (n = 99)	ND (n = 51)	Prev. (%)	OR	95% CI	p-value
Health State						
SOB at rest	5	3	5.3%	0.85	0.20–3.71	0.830
Chronic cough	23	10	22.0%	1.24	0.54–2.86	0.612
Chronic phlegm	9	6	10.0%	0.75	0.25–2.24	0.605
Chronic rhinitis	46	25	47.3%	0.90	0.46–1.78	0.767
Wheeze	32	18	33.3%	0.88	0.43–1.78	0.715
Chest tightness	40	18	38.7%	1.24	0.62–2.50	0.543
Disease State						
Asthma	20	12	21.3%	0.82	0.37–1.85	0.637
Arthritis	36	21	38.0%	0.85	0.44–1.64	0.620
Bronchitis	22	15	24.7%	0.69	0.32–1.48	0.333
Cancer	4	6	6.7%	0.32	0.08–1.18	0.072
Eczema	10	8	12.0%	0.60	0.22–1.64	0.319
Food allergies	8	5	8.7%	0.81	0.25–2.61	0.722
Hay fever	26	20	30.7%	0.55	0.27–1.13	0.103
Other chest	10	4	9.3%	1.32	0.39–4.44	0.652
Pleurisy	7	3	6.7%	1.22	0.30–4.92	0.782
Pneumonia	18	13	20.7%	0.65	0.29–1.46	0.295
Sinusitis	19	16	23.3%	0.52	0.24–1.13	0.095
Pet Ownership						
Cat ownership	37	19	37.3%	1.01	0.50–2.02	0.989
Dog ownership	22	8	20.0%	1.54	0.63–3.74	0.343
Other pet ownership	19	15	22.7%	0.57	0.26–1.25	0.157
Body Mass Index (BMI)						
Underweight: < 18.5	1	0	0.7%	–	–	0.471
Normal: 18.5–24.9	30	20	33.3%	0.67	0.33–1.37	0.273
Overweight: 25.0–29.9	44	16	40.0%	1.75	0.86–3.57	0.122
Obese: > 30.0	24	15	26.0%	0.77	0.36–1.64	0.494
Waist Circumference						
M > 102 cm, F > 88 cm	41	18	39.3%	1.30	0.64–2.61	0.467

n, number of subjects; POS, number of subjects in which anti-IgG antibodies were detected; ND, number of subjects in which anti-IgG antibodies were not detected; Prev, prevalence of risk factor in the entire cohort (n = 150); OR, odds ratio; 95% CI, 95% confidence interval; SOB, shortness of breath.

3.4. Laboratory test results and anthropometric measurements

Findings from the laboratory and anthropometric studies are summarised in Table 4. It was found that IgG seropositive subjects had a significantly lower red cell distribution width (RDW), a measure of the variation in the size and volume of erythrocytes and forms part of the complete blood count (reference range: 11.5–14.6%), than seronegative subjects (average of 3.1 and 5.8, respectively; $p < 0.01$). Also, seropositive subjects had significantly lower absolute eosinophil counts (adult reference range: 4.0–5.5 x 10^6 cells/mL) when compared to the seronegative subjects (average of 3.3 and 4.0, respectively, $p < 0.025$). Furthermore, a significantly higher neutrophil count was observed for seropositive subjects when compared to the seronegative subjects (average of 58.5 and 55.6, respectively, $p < 0.033$). The remaining anthropometric examinations and laboratory results were comparable and no significant association with *T. gondii* seropositivity was found.

When examining for gender, seropositive females where significantly shorter in height (average of 161.5 and 165.4, respectively, $p = 0.025$) and had significantly higher systolic blood pressure (123.0 and 114.5 mmHg, respectively, $p = 0.043$) when compared to their seronegative counterparts. These findings were not found in the male subjects except for creatinine levels where seropositive subjects had significantly higher levels of serum creatinine than seronegative males (98.0 and 89.0 μmol/L, respectively, $p = 0.029$; reference range for males, up to 110 μmol/L).

4. Discussion

We conducted the first seroprevalence study for *T. gondii* infection in a representative Australian population and found high a seroprevalence rate (IgG: 66%) compared to other countries.[3–5,20–26] Comparing our findings to the earlier Australian studies is hindered by earlier focus on congenital cohorts, but lower seroprevalences were found in these studies among pregnant woman throughout the country: 23% in Melbourne,[15] 23% in South Australia,[16] and 26% in Queensland.[16] The only previously published study from Western Australia conducted by Walpole et al.[14] in 1991, investigating the birth prevalence of congenital toxoplasmosis, found that 35% of the tested women were seropositive for anti-*T. gondii* IgG antibodies. Thus, our findings reveal a significant increase in the seroprevalence of *T. gondii* infection in this part of Australia over the last two decades. This high rate of seroprevalence confirms that this disease is widespread among the population which is notable and warrants mitigation.

Conversely, many other countries have observed a decrease in *T. gondii* prevalence over the last 30 years. A 2016 nationwide all-cohort seroprevalence study from Germany[20] reported a lower *T. gondii* IgG seroprevalence (55%; n = 6,564; age: 18–79 years) than previous similar studies among blood donors in North-East Germany between 1994 and 1996 (59%; n = 4,854; age 20–40),[21] and from pregnant women from South-West Germany in 1992 (39%; n = 5,670; age 15–47).[22] Likewise, data from France has shown decreasing *T. gondii* seropositivity, particularly in women of childbearing age, from 54.2% in 1995 to 43.8% in 2003, and 36.7% in 2010.[23] In The Netherlands, *T. gondii* IgG seroprevalence declined from 41% in 1995–1996 to 26% in 2006–2007 (n = 5,541; age 0–79).[24] Furthermore, in the USA from 1999 to 2004, seroprevalence was significantly lower than our study (9.0%; n = 10,477; age 12–49), decreasing from 14.1% in 1988–1994.[26] These differences show that improvements in *T. gondii* infection awareness, monitoring, and prevention in Australia are warranted. Detailed characterisation of Australian national prevalence and risk factors of infection with *T. gondii* is required to adopt universally appropriate prevention measures to avoid exposure and infection. It is well known that being aware of the ways in which *T. gondii* are transmitted and strategies to avoid infection is a key way in which the

Table 4
Association between *Toxoplasma gondii* IgG seropositivity, and selected biochemical and haematological laboratory parameters and anthropometric examinations, raw and after adjustment for gender. BHS, Western Australia.

Parameter	IgG +	IgG -	P	Female IgG + vs IgG-T2DM IgG-		p	Male IgG + vs IgG-CTRL IgG-		p
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Height (cm)	167.8 ± 9.8	169.7 ± 8.2	0.234	161.5 ± 7.9	165.4 ± 6.1	0.025	173.3 ± 7.9	175.3 ± 7.2	0.313
Weight (Kg)	77.1 ± 16.1	77.3 ± 14.5	0.963	68.3 ± 12.6	69.0 ± 11.6	0.787	84.9 ± 14.9	88.1 ± 10.3	0.353
BMI (Kg/m^2)	27.3 ± 4.6	26.8 ± 4.4	0.490	26.2 ± 5.0	25.3 ± 4.7	0.437	28.3 ± 4.0	28.7 ± 3.1	0.669
Waist (cm)	92.3 ± 12.8	89.4 ± 13.9	0.197	84.6 ± 11.2	81.3 ± 9.6	0.186	99.3 ± 9.8	100.1 ± 11.2	0.761
SBP (mmHg)	126.1 ± 17.9	120.8 ± 16.5	0.080	123.0 ± 18.9	114.5 ± 14.5	0.043	128.9 ± 16.7	129.1 ± 15.4	0.949
DBP (mmHg)	78.3 ± 10.7	75.7 ± 9.5	0.140	76.4 ± 9.8	73.5 ± 8.8	0.205	80.0 ± 11.3	78.5 ± 9.7	0.592
Hb (g/L)	145.8 ± 13.2	144.5 ± 12.8	0.547	137.9 ± 10.4	137.8 ± 9.2	0.953	152.8 ± 11.3	153.0 ± 11.7	0.958
Hct (L/L)	0.4 ± 0.0	0.4 ± 0.0	0.666	0.4 ± 0.0	0.4 ± 0.0	0.959	0.4 ± 0.0	0.4 ± 0.0	0.773
RCC[a]	4.7 ± 0.5	4.7 ± 0.4	0.577	4.5 ± 0.4	4.5 ± 0.3	0.991	4.9 ± 0.4	4.9 ± 0.4	0.921
RDW (%)	3.1 ± 5.4	5.8 ± 6.3	< 0.01	3.2 ± 5.3	6.3 ± 6.3	0.027	3.1 ± 5.5	5.3 ± 6.3	0.143
Lymph[a]	30.7 ± 7.8	32.6 ± 7.0	0.140	32.0 ± 8.2	33.7 ± 7.3	0.371	29.5 ± 7.3	31.3 ± 6.5	0.335
Mono[a]	6.9 ± 1.8	7.3 ± 1.4	0.190	6.5 ± 1.8	7.0 ± 1.2	0.210	7.3 ± 1.6	7.7 ± 1.5	0.330
Eosin[a]	3.3 ± 1.5	4.0 ± 1.9	0.025	3.1 ± 1.5	4.0 ± 2.2	0.054	3.5 ± 1.4	4.0 ± 1.5	0.220
Baso[a]	0.6 ± 0.5	0.5 ± 0.5	0.133	0.5 ± 0.5	0.3 ± 0.5	0.100	0.7 ± 0.5	0.7 ± 0.5	0.950
Neutro[a]	58.5 ± 7.9	55.6 ± 7.6	0.033	58.0 ± 8.6	55.0 ± 8.1	0.142	59.0 ± 7.4	56.4 ± 7.0	0.160
Platelets	244.4 ± 62.1	254.5 ± 61.0	0.348	271.6 ± 57.6	266.8 ± 52.8	0.723	220.4 ± 56.3	238.9 ± 68.1	0.229
MCV (fL)	89.7 ± 4.0	90.0 ± 3.9	0.699	89.2 ± 3.2	89.1 ± 3.3	0.990	90.2 ± 4.5	91.0 ± 4.4	0.468
TP (g/L)	74.8 ± 3.9	74.2 ± 3.5	0.413	74.3 ± 3.5	74.0 ± 3.7	0.790	75.2 ± 4.2	74.5 ± 3.3	0.482
Alb (g/L)	45.5 ± 2.6	46.3 ± 1.9	0.055	45.7 ± 2.3	45.9 ± 1.9	0.716	45.3 ± 2.9	46.8 ± 1.8	0.025
Bili (μmol/L)	10.8 ± 6.0	9.2 ± 3.7	0.087	8.9 ± 4.1	8.6 ± 3.3	0.764	12.5 ± 6.9	10.0 ± 4.1	0.124
Gluc (mmol/L)	5.2 ± 0.5	5.1 ± 0.4	0.223	5.0 ± 0.4	5.0 ± 0.4	0.654	5.3 ± 0.5	5.3 ± 0.3	0.465
Insulin (mU/L)	8.1 ± 6.0	6.9 ± 4.2	0.175	7.0 ± 4.1	6.0 ± 2.9	0.248	9.1 ± 7.1	8.0 ± 5.2	0.500
CRP (mg/L)	2.6 ± 2.8	2.4 ± 3.7	0.770	2.6 ± 3.5	2.1 ± 2.0	0.483	2.5 ± 2.1	2.8 ± 5.3	0.742
Chol (mmol/L)	5.5 ± 0.9	5.4 ± 1.0	0.400	5.6 ± 1.2	5.4 ± 1.0	0.316	5.4 ± 1.0	5.3 ± 1.1	0.710
HDL mmol/L)	1.6 ± 0.5	1.6 ± 0.5	0.898	1.8 ± 0.5	1.7 ± 0.4	0.306	1.4 ± 0.4	1.4 ± 0.5	0.887
LDL (mmol/L)	3.3 ± 0.8	3.2 ± 0.9	0.624	3.3 ± 0.9	3.2 ± 0.9	0.727	3.3 ± 0.9	3.3 ± 1.0	0.772
Trig (mmol/L)	1.4 ± 0.7	1.3 ± 0.5	0.230	1.2 ± 0.5	1.1 ± 1.4	0.418	1.6 ± 0.7	1.5 ± 0.7	0.586
ALP (U/L)	66.8 ± 19.1	70.0 ± 25.7	0.390	63.1 ± 19.1	67.4 ± 25.2	0.407	70.0 ± 17.8	73.5 ± 26.6	0.524
ALT (U/L)	14.2 ± 7.8	13.7 ± 6.4	0.736	12.6 ± 7.6	13.0 ± 6.7	0.811	15.5 ± 7.7	14.7 ± 6.1	0.659
Creat (μmol/L)	77.7 ± 20.3	76.0 ± 14.6	0.766	61.1 ± 9.0	66.3 ± 9.0	0.179	98.0 ± 5.9	89.0 ± 9.5	0.029

+, anti-IgG antibodies not detected; -, anti-IgG antibodies not detected SD, standard deviation; BMI. Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; mmHg, millimeters of mercury; Hb, haemoglobin; Hct, haematocrit; RCC, red cell count; RDW, red cell distribution width; WCC, white cell count; MCV, mean cell volume; TP, total protein; Alb, albumin; Bili, bilirubin; Gluc; glucose; Chol, cholesterol; Trig, triglycerides; CRP, c-reactive protein; HDL, high-density lipoproteins: LDL, low-density lipoproteins; ALP, alkaline phosphatase; ALT, alanine transaminase; Creat, creatinine.

[a] Red cell, lymphocyte, monocyte, eosinophil, basophil, and neutrophil counts are recorded as absolute counts (x10^6 cells/mL).

infection can be mitigated.[20] Because toxoplasmosis is not a notifiable disease in Australia, information on national disease incidence from the general population is missing. As discussed above, previous serosurveys for *T. gondii* are limited with respect to representativeness and sample sizes. Available reports are based on convenience sampling, mainly from pregnant females, and lack a randomised participant selection protocol. We therefore recommend Australian health authorities consider toxoplasmosis as a notifiable disease to: improve education and raise awareness; lower the number the frequency of primary infections; and commence longitudinal epidemiological data collection. In Germany, congenital toxoplasmosis is a mandatory notifiable disease to the Robert Koch Institute which implements data collection and processing of case data for the German infectious disease notification system.[20]

The observed IgG seroprevalence increased at an average rate of 0.8% with each year of age. A similar rate of 1.09% has been previously reported from Germany.[20] Seropositivity increased rapidly in all age groups remaining at over 70% in the 65–74, 75–84, and > 84 year-old age groups. High seroprevalence in these groups is particularly problematic since immunosuppression becomes more prominent with age. With reference to gender, men had a 1.59-times higher chance of being IgG seropositive. Since Australian males consume almost 50% more processed meat than females,[26] and several studies have identified consumption of undercooked or raw meat and meat products as risk factors for foodborne transmission to humans, the higher seroprevalence observed in men in the current study may be partly explained by certain eating habits. In particular, kangaroo meat has been recognised as a source of *T. gondii* infection in Western Australia and due to its popularity as a lean low-fat meat, it is usually served rare to humans and raw as pet meat.[27] *Toxoplasma gondii* bradyzoites remain infective when meat is undercooked, making ingestion a risk factor.[27] Robson et al.[28] reported an outbreak of 13 cases in Australia that were attributable to the consumption of undercooked kangaroo meat. With respect to the seroprevalence of *T. gondii* IgM antibodies, it is difficult to draw conclusions due to the low numbers of seropositive subjects in the sampling of the current study.

In the present study, ownership of cats, dogs, or other pets was not identified to be a risk factor associated with *T. gondii* seropositivity. Previous epidemiological studies have reported similar observations.[29,30] Although the lack of an association of cat ownership may be surprising, due to the cat family being biologically essential to the life cycle of *T. gondii* as the only definitive hosts, contact with cats appears to be a less important risk factor when compared to other well established risk factors such as contact with contaminated foods.[20] While data from other studies support cat contact as a risk factor,[31–33] prevention of *T. gondii* infection via cat exposure may be possible as cats only shed oocysts for up to three periods in a lifetime. In addition, oocyst sporulation can be avoided by regular removal of cat litter.[34] Hence, the results from the current study may indicate that Australian cat owners are maintaining the hygiene of their animals safely.

Although previous studies have identified some factors such as BMI and some diseases like cancer as risk factors associated with the infection with *T. gondii*,[20,31,32,35] the results of the present study did not find any association between the selected respiratory and chest conditions; various other disease states; anthropometric and body composition measurements, and *T. gondii* seropositivity in Busselton, Western Australia. With respect to the biochemical and hematological

laboratory analyses, all parameters except red cell distribution width (RDW) were typically normal and no association with *T. gondii* seropositivity was found among the cohorts of the present study. Significantly higher RDW values were found in seronegative subjects when compared to seropositive subjects ($p < 0.01$). The RDW forms part of the standard laboratory full blood count measuring the range of variation in red blood cell width and recently, higher values have been associated with various disease states including various forms of cancer[36]; carotid artery atherosclerosis[37]; and metabolic syndrome.[38] Moreover, Patel at al.[39] conducted a mortality follow up study of 8,175 adults whose RDW values had been previously recorded. They found that higher RDW values were associated with an increased risk of death and RDW is a strong predictor of mortality. Further investigation is warranted to confirm our findings, and to explore the causes of lower RDW values from seropositive subjects. Likewise, the significantly lower absolute eosinophil counts observed in the seropositive subjects when compared to the seronegative subjects (average of 3.3 and 4.0, respectively, $p < 0.025$) should be investigated further. Increased eosinophil count (eosinophilia) is a hallmark of parasitic infections[40] and *T. gondii* has been shown to cause mild eosinophilia.[41]

The strengths of the present study included a systematic health screening process leading to a characterisation of a representative study population including adjustment for gender and age. The analyses of the present study were based on diagnostic seroprevalence data, however, seroconversion is not equivalent to clinical presentation of the disease. This weakness should be considered in future studies. Lastly, specific environmental variables like lifetime cat exposure or lifetime undercooked meat consumption, that were not available from the BHS survey, should also be considered in future studies.

5. Conclusion

Toxoplasma gondii infection in Western Australia is prevalent. Pet ownership, amongst other variables, was not identified as significant risk factor associated with *T. gondii* infection in both male and female subjects. *Toxoplasma gondii* infection has been neglected in Australian notifiable disease programs therefore Australian public health authorities should focus on improving education to raise awareness of *T. gondii* and commence longitudinal epidemiological data collection to contribute and supplement public health models targeting *T. gondii* transmission control.

Ethics approval and consent to participate

approved by the Human Research Ethics Committee of Edith Cowan University on 31 January 2017, approval number: 16090. Written informed consent was obtained from the study participants.

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Declaration of competing interest

The authors declare no conflict of interest.

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