

## **Risk factors for *Toxoplasma gondii* seropositivity in the Old Order Amish**

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**Data Availability:** The data cannot be shared because of the original protocol and Amish consent. A collaborative effort could be considered and interested individuals should contact the corresponding author.

**40-word summary:** Risk of infection with the major foodborne pathogen, *Toxoplasma gondii*, varies by individual, geographic, sociocultural, and economic factors. We assessed the risk of toxoplasmosis, focusing on food safety and environmental factors, among the Old Order Amish, a relatively homogenous population.

## Summary

*Toxoplasma gondii* is an important human disease-causing parasite. In the US, *T. gondii* infects >10% of the population, accrues economic losses of US\$3.6 billion/year, and ranks as the second leading culprit of foodborne illness-related fatalities. We assessed toxoplasmosis risk among the Old Order Amish, a mostly homogenous population with high prevalence of *T. gondii* seropositivity, using a questionnaire focusing on food consumption/preparation behaviours and environmental risk factors. Analyses were conducted using multiple logistic regression. Consuming raw meat, rare meat, or unpasteurized cow or goat milk products were associated with increased odds of seropositivity [unadjusted ORs: 2.192, 1.613, 1.718, and 1.741, respectively]. In separate models by sex, consuming raw meat, or consuming unpasteurized cow or goat milk products, were associated with increased odds of seropositivity among women; washing hands and touching meat with decreased odds of seropositivity among women [adjusted OR (AOR): 0.462]; and cleaning cat litterbox with increased odds of seropositivity among men (AOR: 5.241). This is the first study to assess associations between behavioural and environmental risk factors and *T. gondii* seropositivity in a US population with high seroprevalence for *T. gondii*. Our study emphasizes the importance of proper food safety behaviours to avoid risk of infection.

## INTRODUCTION

The apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) infects one-third of the world's population, disproportionately affects socioeconomically disadvantaged groups, and ranks among five neglected parasitic infections targeted in the United States (US) by the Centers for Disease Control (CDC) for public health action[1, 2]. Some estimates indicate that in the US ~170,000 new toxoplasmosis cases occur annually and that 1.1 million are currently infected, amounting to infection among 13.2% (age-adjusted: 12.4%) of the population [3]. Other sources note that although >40 million men, women, and children in the US may harbour the parasite without symptoms because the immune system prevents occurrence of the full-blown illness, consequences of infection can be severe for the immunocompromised and for women infected during or shortly before pregnancy [2].

The burden of *T. gondii* infection by foodborne routes, which is estimated to account for about half of all US cases, exceeds \$3.6 billion and equates to the loss of 459,481 quality-adjusted lifedays (QALDs)[4]. According to Scallan et al. (2011), 2.6% of US-acquired foodborne *T. gondii* infections will result in hospitalizations, and 0.02% will result in death, leading to an estimated 327 deaths annually, making toxoplasmosis the second leading cause of foodborne illness fatality in the US [5].

Sexual reproduction of *T. gondii* occurs in its definitive felid hosts, including cats, while asexual reproduction occurs in intermediate hosts, including humans and nearly all warm-blooded vertebrates. The parasite has three infectious stages[6]<sup>15</sup>: sporulated oocysts that contain sporozoites, tachyzoites that invade and multiply rapidly within the

cells of intermediate hosts, and bradyzoites that slowly replicate in tissue cysts (life cycle detailed in Figure 1). Tissue cysts are most often found in the central nervous system; the eyes; and smooth, skeletal, or cardiac muscles. While bradyzoites in tissue cysts characterize chronic infection, tachyzoites characterize primary acute or reactivated infections, which can trigger adaptive IgG immune responses[7, 8]. In reactivation, tissue cysts rupture and release bradyzoites that transform back into destructive tachyzoites<sup>11,13</sup>. Toxoplasmosis may present in immunocompetent individuals with painless lymphadenopathy; mononucleosis-like symptoms, including fever, malaise, sore throat, and maculopapular rash; ocular infections in some older populations; or no symptoms in pregnant women[9]<sup>12</sup>. Severe clinical manifestations, such as pneumonitis, chorioretinitis, or multi-organ involvement, occur most often in reactivated and primary infections among immunocompromised individuals and in congenital infections[9, 10]. HIV-positive individuals often present with life-threatening toxoplasmic encephalitis, characterized by headache, confusion, weakness, focal neurologic involvement, and seizures[11, 12]. Previously, chronic infections were not associated with clinical manifestations[9], but recent studies have linked chronic infection to schizophrenia[13-15] and suicidal behaviour [16, 17].

Multiple behavioural, socioeconomic, and demographic factors may contribute to the risk of toxoplasmosis. Studies focusing on behavioural risk factors often use a combination of immunoassays to determine infection and questionnaires to conduct epidemiologic assessment of potential exposures. While the questionnaires themselves are often tailored to specific populations, many ask similar questions regarding behaviour-related exposures: most notably, eating different meats[18-22] (porcine,

bovine, lamb/mutton, other); handling or eating raw or undercooked meat[18, 22]; consuming dairy/ dairy products[22-24]; eating unwashed or improperly washed fruits and/or vegetables[22, 25, 26]; gardening and/or engaging in contact with soil[18, 21, 22, 26]; living with pets, such as cats and/or dogs at home[18, 19, 26]; drinking water (treatment, source, other characteristics)[19, 22, 27]; and even hunting/consuming wild game (venison, boar, other)[18, 27]. In the US, those below the federal poverty threshold have higher *T. gondii* seropositivity[3], while higher early-life socioeconomic status (SES) has been shown to be related to seronegativity[28]. Other risk factors for higher seroprevalence, specifically in the US, include foreign country of birth, Hispanic and non-Hispanic Black race/ethnicity, lower than high school education levels, crowded housing conditions, rurality/urbanicity, and employment type[3, 22].

The Old Order Amish (OOA) population in the US, by virtue of its homogeneous characteristics, provides a unique opportunity to assess relationships between *T. gondii* seropositivity and specific behavioural and environmental (including food-related) risk factors. *T. gondii* seropositivity prevalence levels among the OOA exceed those found among the general US population[29], and the geographic, ethnic, and socioeconomic homogeneity of the OOA population [30] helps to control for potential confounding that can otherwise complicate risk factor assessment. In addition, the Amish lifestyle presents the prospect of evaluating potential associations between *T. gondii* seropositivity and culturally specific meal-preparation practices, such as slaughtering, smoking, curing, and canning meats[31]. These distinctive characteristics, in conjunction with antibody testing and data collection using OOA lifestyle-specific questions, allowed this study to pursue the objective of advancing understanding of

relationships between the prevalence of *T. gondii* seropositivity and behavioural and environmental (including food-related) risk factors of infection, while minimizing confounding by other potential sociodemographic and behavioural determinants.

## **METHODS**

### STUDY POPULATION

#### THE AMISH WELLNESS STUDY

The Amish Wellness Study (AWS), an ongoing community-based investigation conducted by the University of Maryland Baltimore and approved by the University of Maryland Baltimore Institutional Review Board, was established in 2010 to offer health screening and collection of DNA, blood samples, and basic medical information to address specific research questions. Criteria for participation included membership in the OOA community of Lancaster County, Pennsylvania; age of  $\geq 18$  years; and an informed consent. A registered nurse obtained multiple clinical measures of general health (e.g., weight, height, waist and hip circumference, blood pressure, measures of respiratory health). An Amish liaison facilitated administration of the study questionnaire in person to assess several aspects of each participant's health and well-being, including mood, sleep, and medical, personal and family histories; as well as to evaluate life-style factors. A University of Maryland Amish Research Clinic research nurse confirmed completion of each questionnaire. A fasting blood sample was drawn for measurement of lipids and glucose, and from this sample, we also measured *T. gondii* IgG antibodies for this study.

For the present study, we sent the Amish *Toxoplasma* Risk Factor Questionnaire (ATRFQ) to a convenience sample from the AWS (n = 3551, nonrandomly selected, based on availability to participate), between December 30, 2015 and January 3, 2016. We evaluated participant responses to the ATRFQ, along with their demographic information and *T. gondii* antibody results from the AWS.

#### IMMUNOGLOBULIN G SEROINTENSITY AND SEROPOSITIVITY TESTING

For this study, the laboratory of Maureen Groer at the University of South Florida in Tampa determined *T. gondii* antibody seroprevalence using the enzyme-linked immunosorbent assay (ELISA) (IBL International, Männedorf, Switzerland) that tests for RH factor common to all *T. gondii* serotypes. Concentrations of  $\geq 12$  IU/ml were defined as seropositive, and of 8-12 IU/ml as equivocal. The equivocal samples were tested a second time, and if the second testing yielded samples that tested positive for concentrations of  $\geq 12$  IU/ml on the second test were also considered positive as previously reported [32].

#### AMISH *TOXOPLASMA* INFECTION RISK FACTOR QUESTIONNAIRE (ATRFQ)

The ATRFQ was developed by researchers at the University of Maryland Baltimore with input from epidemiologists at FDA's Center for Food Safety and Applied Nutrition (CFSAN) and co-author Jeffrey Jones, based on current risk factor literature and subject matter expertise. The questionnaire was adapted after incorporating input from 3 sessions of review and feedback by a group of Amish liaisons, 3 sessions of feedback by nurses experienced in working with the Amish, and individual researcher comments and questions to maximize comprehension and cultural sensitivity. The



questionnaire consisted of 30 questions focusing primarily on food consumption and preparation, as well as other environmental risk factors, such as water sources, pet ownership and contact, and contact with soil. The pet component specifically asked about cat/ kitten ownership, cat breeding, cat litter cleaning, where the cats live (outdoors vs indoors), where the cats/ kittens eat, type of food they eat, visits of not-owned cats, type of cats seen in the garden or yard, cats for breeding, feral cats (domesticated and returned to the wild), and stray cats (lost or abandoned). . It was mailed to study participants between December 30, 2015 and January 3, 2016 with a cover letter, response envelope, and US\$2 compensation. The cover letter informed participants that they could keep the \$2 even if they decided not to participate. Responses from the returned questionnaire received by April 11, 2016 were entered into an Access database. Returned envelopes received after April 11, 2016 were not included in the analytical sample used for this study.

## STATISTICAL ANALYSES

The analytical sample included those with both *T. gondii* serological measures and ATRFQ responses. Analyses were performed to describe the analytical sample (Table 1) and to assess the strength and significance of associations with examined behavioural and environmental (including food-related) risk factors through models estimating odds of *T. gondii* seropositivity (Tables 2-4). In addition to age and sex derived from the parent AWS survey, the ATRFQ variables addressed contact with soil, pets (including cats and litter boxes), and shoes/feet before touching food; farm vs. other-than-farm residence and time at residence; drinking untreated or treated water and drinking water source/filtration; working with animals/raw meat/horses; consuming

locally-produced, cooked, cured, frozen, canned, raw and/or rare meats; consuming raw oysters; consuming unpasteurized goat and/or cow milk/products; washing produce; washing hands/utensils after raw meat contact; and eating outside the home.

Bivariate analyses, including chi-square ( $X^2$ ) and t-tests, compared seropositive to seronegative to identify significant or borderline significant ( $P$  value  $<0.05$  and  $<0.20$ ) variables. For those variables, we then specified comparison reference groups and estimated unadjusted odds ratios (UORs), 95% confidence intervals (CIs), and  $P$  values (Tables 2 and 3). We included the variables that exhibited significance in the bivariate UOR analysis ( $P$  value  $<0.05$ ) in the final unconditional multiple logistic regression models by sex to estimate adjusted odds ratios (AORs), 95% CIs, and  $P$  values, evaluating the associations between each of the predictor variables and the outcome of *T. gondii* seropositivity as defined above (Table 4). These analyses were performed using SAS 9.4 (Cary, NC), as well as the  $X^2$  calculator in McDonald (2014)[33]. We also adjusted the results for relatedness (heritability) and shared households by applying a variance component approach using SOLAR 6.6.2 software (San Antonio, TX) to assess significance ( $P$  value  $<0.05$ ) [34].

## RESULTS

Of the 3551 questionnaires mailed to study participants, 1518 (43%) were returned. *T. gondii* serology was assessed in 55.5% ( $n=843$ ) of those who returned questionnaires. We limited our analytical sample to the 843 Amish subjects with both serology test results and questionnaire response data (23.7% of the 3551 who were mailed and returned the questionnaire also had serology results).

More than half (56.7%) of the 843 study participants tested positive for *T. gondii* IgG antibodies (Table 1). Nearly two-thirds of participants (60.1%) were female among both the seropositive and seronegative groups. The age distribution/n was similar among seropositive individuals, but not among seronegative individuals or the whole study sample. Mean and median ages were higher among seropositive than among seronegative individuals, and there were more seropositive than seronegative males.

In addition to sex, the  $X^2$  test results from the first step of the bivariate analysis indicated significant/borderline significant associations between *T. gondii* seropositivity ( $P$  values  $<0.20$ ) and the category-based variables capturing farm vs. other-than-farm residence and time at residence; working with horses; consuming unpasteurized cow milk/products; consuming cured, rare, and raw meats; pet contact; and washing hands/utensils after raw meat contact, as well as the frequency-based variables addressing how often unpasteurized cow milk/products, raw meats, rare meats, and meals outside the home were consumed.

Of those variables, the UOR estimates from the second step of the bivariate analysis showed significance ( $P$  value  $<0.05$ ) for pet contact, consumption of meat and unpasteurized milk/products, and handwashing behaviours (Table 2), as well as for frequency of consumption of unpasteurized cow milk/ yoghurt, rare meat, raw meat, and meals outside the home (Table 3).

The four meat-eating behaviours, as well as the two variables addressing the frequency of consumption, were all associated with *T. gondii* seropositivity. The odds of seropositivity from eating rare and raw meat compared to not eating rare and raw meat

were 1.613 (95% CI: 1.017, 2.560) and 2.192 (95% CI: 1.172, 4.101), respectively, while seropositivity among those who reported eating compared to not eating raw ground beef was greater (OR: 2.568, 95% CI: 0.996, 6.623). Only one individual reported eating raw ground pork and was seropositive. Odds of seropositivity comparing consumption of unpasteurized milk to not consuming raw milk was 1.211 (OR: 1.211, 95% CI: 1.055, 1.391). Among the behaviours related to pets, only cleaning a cat's litterbox was found statistically significantly associated with *T. gondii* seropositivity (OR: 2.451, 95% CI: 1.167, 5.143).

Because of the significant difference found between sex and seropositivity in the bivariate analysis, separate models by sex were used to estimate the adjusted associations between the other variables also identified as significantly associated with seropositivity (Table 4). Several of the associations between *T. gondii* seropositivity and behavioural and environmental (including food-related) risk factors did not retain significance in the adjusted models by sex. However, the separate adjusted models by sex showed that significance persisted nominally for cleaning the litterbox (among men only), eating raw meat (among women only), and washing hands after touching raw meat (protective in women only). Consuming raw goat or cow milk was significantly associated with seropositivity among women only (OR: 1.863, 95% CI: 1.086, 3.197; OR: 1.930, 95% CI: 1.127, 3.304, respectively, where only 2 respondents indicated consumption of unpasteurized goat milk products, and 421 indicated intake of unpasteurized cow milk products). Washing versus not washing hands after touching raw meat was also significantly associated with seropositivity among women only (OR:

0.462, 95% CI: 0.260, 0.823). These adjusted significant results held, even after adjustment for heritability and shared household.

## DISCUSSION

This study found that members of the OOA community of Lancaster, Pennsylvania have a high seroprevalence of *T. gondii* antibodies, with over 56% of study participants testing seropositive, exceeding the levels reported in many other US populations, although similar to levels reported in Java, Indonesia (62.5%)[24] and among the Nanvik Inuit of Canada (59.8%)[27]. The Jones et al. (2014) US-based analysis of the 2009-2010 National Health and Nutrition Examination Survey (NHANES) that included a wave of participants aged  $\geq 6$  years found a much lower seroprevalence of 13.2% [95% confidence limit (CL): 11.8%, 14.5%] prior to adjustment for age and an age-adjusted seroprevalence of 12.4% (95% CL: 11.1%, 13.7%)[3]. As these studies vary in terms of recruitment and analytical methodologies, we suggest caution when interpreting these differences. The uniqueness of our study population may further complicate comparisons—as previously mentioned, the OOA community is an ethnically, behaviourally, and culturally distinct group. We are not aware of any studies assessing the seroprevalence of *T. gondii* in other Amish populations and have found only one study focusing on a similar population. Alvarado-Esquivel et al. (2010) conducted a *T. gondii* risk factor and seroprevalence analysis among 152 Mennonites in Durango, Mexico, an isolated and mostly rural population of ethnic Germanic descent, with cultural and behavioural aspects that differ from the surrounding communities[35]. They found lower seroprevalence (30.3%) among the Mennonites of Durango[35], compared to the 56% seroprevalence rate that we have observed in the OOA

community of Lancaster, Pennsylvania. Also, the seroprevalence of *T. gondii* IgG antibodies in the general population of Durango City, Mexico was reported to be 6.1%, which was remarkably less than the seroprevalence in the Mennonite community living in the same city[36]. This difference in seroprevalence may relate to diverse factors, including food sources, diet, unique group practices, geographic characteristics, and other factors that differ between this Mennonite community and the OOA.

The rural location of residence may contribute to the higher seroprevalence among the OOA compared to other populations. Seroprevalence studies often consider living in a rural environment a risk factor because of the presence in rural settings of potentially infected animals that can transmit the parasite to humans in a variety of ways[12]. The finding of higher seroprevalence among rural compared to urban populations is consistent with the results from the United Kingdom, Germany, and Ireland[21, 37, 38]. In addition, a 2013 study by Muñoz-Zanzi et al. found that children residing on farms in Wisconsin were more likely seropositive than those who did not reside on farms[39]. In their multivariate analysis of the 1988-94 wave of NHANES, Jones et al. 2001 reported significantly lower seropositivity among non-Hispanic Blacks living in metropolitan areas (>1 million population) (OR: 0.74, 95% CI: 0.59, 0.93) than among non-Hispanic Blacks living in non-metropolitan areas[40]. Yet, the risk of *T. gondii* infection was not different among residents of metropolitan and non-metropolitan areas for populations of non-Hispanic Whites or Mexican-Americans[40]. Our study population lacked a proper urban counterpart to assess the impact of rurality/urbanicity, so we cannot exclude the possibility that there is simply high seroprevalence in the general study area.

Several of the food- and food safety-related risk factors found significantly associated with *T. gondii* seropositivity in this study are consistent with results from other studies. First, consumption and handling of raw and/or undercooked meats was associated with *T. gondii* seropositivity for both beef and pork, as previously seen in the literature[18, 21-26]. While the practice of cleaning a cat's litterbox was also observed to be a risk factor among males in our study, our findings highlight the importance of the foodborne transmission route for *T. gondii*. In addition, washing hands was found protective of *T. gondii* seropositivity in our study, especially among women. Our findings emphasize the importance of proper food safety behaviours, such as cooking meat to a safe temperature prior to eating/ not consuming raw meat, which is effective at diminishing risk of contracting the parasite. Also, people should avoid accidental contamination by thoroughly washing hands before and after handling meats, and properly washing preparation surfaces and utensils (such as knives) with soap and hot water after use. Washing of hands and/or use of gloves during gardening, may also help prevent contamination[2]. Our study also found that consumption of unpasteurized milk products was associated with risk of seropositivity. While intake of these products provides a possible route of exposure to the parasite[22-24], consumption rates are low among the US population[22, 41, 42], and thus could explain, in part, the increased seropositivity among the Amish.

This study provided novel insights into risk factors for infection with a highly prevalent parasite in a unique US population. Among several strengths, the study population is known for its ethnic and socioeconomic homogeneity, which may have helped to minimize confounding by unmeasured variables. Second, response rates

were high for both the AWS questionnaire and the ATRFQ. Third, to counteract the possibility that the participants' close family and household aggregation could have produced spurious associations, the study adjusted post-hoc for heritability and household in the analytical sample, without any loss of statistical significance for any of the positive findings reported in our study.

Among the study's important limitations, the findings may not be generalizable to other populations in the US or elsewhere, given the unique characteristics of the culturally distinct OOA[30]. In addition, we saw different distributions between age groups, which may be due to cumulative seropositivity and may have biased some of the observed associations. Our study was also only included adults (age 18y or older), which means that we are unable to assess whether infections were acquired during childhood. Consequently, we are unable to assess certain risk factors and are also unable to provide recommendations to prevent these potential exposures. This study is also limited by a relatively small sample size given the number of risk factors analysed. Although the high seropositivity in the OOA may have mitigated sample size issues, the low incidence of certain behaviours may have masked the statistical significance of their potential association with seropositivity.

We presented our findings, both formally and informally, to the OOA community in Lancaster, PA. We discussed the different *T. gondii* risk factors identified in our analysis and specifically addressed the observed sex-based differences. Direct feedback from the OOA community and the findings themselves have and will continue to be used to develop content for future education efforts, including food safety. We plan on applying for grants to develop additional educational materials, train Amish



educators, and conduct future studies to assess the possible relationships between knowledge and practice among the OOA. For example, future research efforts include developing a study to assess *Toxoplasma* risk factors and foetal infection, morbidity, and mortality, and possibly establishing a longitudinal study to look at a variety of other important genetic, epigenetic, proteomic, clinical, and environmental factors, with sampling data for *T. gondii* in food, soil, water, and farm and domestic animals, as well as neuroimaging and other markers of physical and neuropsychiatric health and functioning.

## CONCLUSION

This study found *T. gondii* seropositivity among more than half of the participants from a unique OOA community with culturally specific meal preparation practices. The study highlights significant behavioural and environmental (including food-related) risk factors associated with infection, such as consuming undercooked meats and unpasteurized cow milk products, as well as protective behaviours, such as eating meals outside of the home and washing hands after contact with raw meat, showing the importance of proper food safety practices for meal preparation, which is of growing relevance to the consumers in the current general US population, as well. Future research will focus on characterizing specific microorganism traits, including serotypes, markers of infection, and genetic, physiological, and clinical associations with *T. gondii* seropositivity, as well as advancing understanding of education and outreach needed to

promote protective behaviours and reduce the burden of toxoplasmosis from foodborne and other routes.

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**Table 1. Demographic Characteristics of Old Order Amish Participants, *Toxoplasma gondii* Serostatus and Risk Factor Study, 2015-2016.**

| Characteristic   | Total<br>n (%)<br>843 (100%) | Seropositive<br>n (%)<br>478 (56.7) | Seronegative<br>n (%)<br>365 (43.3) | P value* |
|--|------------------------------|-------------------------------------|-------------------------------------|----------|
| <b>Age (y)</b>   |                              |                                     |                                     |          |
| 18-29  | 180 (21.4)                   | 77 (16.1)                           | 103 (28.2)                          | <0.0001  |
| 30-39  | 145 (17.2)                   | 77 (16.1)                           | 68 (18.6)                           |          |
| 40-49  | 153 (18.2)                   | 90 (18.8)                           | 63 (17.3)                           |          |
| 50-59  | 138 (16.4)                   | 75 (15.7)                           | 63 (17.3)                           |          |
| 60-69  | 136 (16.1)                   | 92 (19.3)                           | 44 (12.1)                           |          |
| 70+  | 91 (10.8)                    | 67 (14.0)                           | 24 (6.6)                            |          |
| Mean (SD)  | 46.6/45.0 (16.8)             | 49.3/49.0 (16.7)                    | 43.2/41.0 (16.3)                    |          |
| <b>Sex</b>   |                              |                                     |                                     |          |
| Female   | 507 (60.1)                   | 262 (54.8)                          | 245 (67.1)                          | <0.0003  |
| Male   | 336 (39.9)                   | 216 (45.2)                          | 120 (32.9)                          |          |
| <b>Body mass index</b>   |                              |                                     |                                     |          |
| Mean (SD)  | 26.5 (4.9)                   | 26.7 (5.0)                          | 26.3 (4.8)                          | 0.24     |
| <b><i>T. gondii</i> titre concentration (IU/ml) **</b>   |                              |                                     |                                     |          |
| Median (SD)  | 15.6 (3.4, 77.2)             | 67.9 (24.6, 113.6)                  | 2.8 (1.5, 4.9)                      | <0.0001  |
| SD, standard deviation; <i>T. gondii</i> , <i>Toxoplasma gondii</i> .  |                              |                                     |                                     |          |
| *P value from $\chi^2$ test of independence for age and sex[33]; from SAS 9.4 (Cary, NC) for t-test; italics denote significance at P <0.05. |                              |                                     |                                     |          |
| ** <i>T. gondii</i> titre concentration: seropositive $\geq 12$ IU/ml; seronegative <12 IU/ml.   |                              |                                     |                                     |          |

**Table 2. Bivariate Analysis of Unadjusted Associations (Unadjusted Odds Ratios) of Serostatus and Category-based Behavioural Risk and Protective Factors of Old Order Amish Participants, *Toxoplasma gondii* Serostatus and Risk Factor Study, 2015-2016.**

| Behavioural Risk or Protective Factor   | Seropositive of those positive for behaviour<br>n/n (%) | Seropositive of those negative for behaviour<br>n/n (%) | Unadjusted Odds Ratio | 95% CI | P value     |              |
|---|---|---|-----------------------|--------|-------------|--------------|
| <b>Pets</b><br>(ref: no pets)   | Has pets  | 340/622 (54.7)  | 127/204 (62.3)        | 0.959  | 0.670-1.372 | 0.818        |
|   | Has cats  | 267/455 (58.7)  | 211/388 (54.4)        | 1.209  | 0.912-1.603 | 0.187        |
|   | Has kittens   | 164/287 (57.1)  | 314/556 (56.5)        | 1.096  | 0.811-1.481 | 0.549        |
|   | Has contact with cats                                   | 364/638 (51.1)  | 114/205 (55.6)        | 1.134  | 0.818-1.571 | 0.452        |
|   | Cleans the litterbox                                    | 30/40 (75.0)  | 396/703 (56.3)        | 2.451  | 1.167-5.143 | <i>0.018</i> |
| <b>Meat consumption</b><br>(ref: no consumption)  | Ate rare meat   | 62/95 (65.3)  | 416/748 (55.6)        | 1.613  | 1.017-2.560 | <i>0.042</i> |
|   | Ate raw meat  | 57/96 (59.4)  | 421/747 (56.4)        | 2.192  | 1.172-4.101 | <i>0.014</i> |
|   | Ate raw ground pork*                                    | 1/1 (100.0)   | 471/836 (56.3)        | ∞      | ∞           | <0.05*       |
|   | Ate rare ground beef                                    | 19/25 (0.76)  | 459/818 (56.1)        | 2.568  | 0.996-6.623 | 0.051        |
| <b>Unpasteurized milk consumption**</b><br>(ref: no consumption)  | Consumed unpasteurized cow or goat milk products*       | 423/735 (57.6)  | 55/108 (50.9)         | 1.718  | 1.116-2.643 | <i>0.01</i>  |
| <b>Washing hands</b><br>(ref: not always washing hands after touching raw meat)   | Always washing hands after touching raw meat            | 348/636 (54.7)  | 80/125 (64.0)         | 0.642  | 0.426-0.950 | <i>0.035</i> |
| <p>*Only 1 respondent indicated raw ground pork intake.</p> <p>**Only 2 respondents indicated consumption of unpasteurized goat milk products; 421 indicated intake of unpasteurized cow milk products.</p> <p>Italics denote significance at <math>P &lt; 0.05</math>.</p> |   |   |                       |        |             |              |

**Table 3. Bivariate Analysis of Associations (Unadjusted Odds Ratios) of Serostatus and Frequency-based Behavioural Risk and Protective Factors of Old Order Amish Participants, *Toxoplasma gondii***

| Behavioural risk or protective factor          |                                 | Never                         | <1/week      | ≥1 week                     | Daily           | Unadjusted odds ratio        | 95% CI        | <i>P</i> value        |
|--|---------------------------------|-------------------------------|--------------|-----------------------------|-----------------|------------------------------|---------------|-----------------------|
| <b>Consumption of:</b>                         | Unpasteurized cow milk/ yoghurt | 57/112 (50.9)                 | 47/82 (64.0) | 77/131 (58.8)               | 297/ 518 (57.3) | 1.211                        | 1.055-1.391   | <i>0.007</i>          |
|  | Rare meat                       | 416/748 (55.6)                | 14/20 (70.0) | 2/3 (66.7)                  | 1/1 (100.0)     | 1.434                        | 1.023-2.011   | <i>0.037</i>          |
|  | Raw meat                        | 440/790 (55.7)                | 2/3 (66.7)   | 1/1 (100.)                  | 0/0 (n/a)       | 2.003                        | 1.120-3.584   | <i>0.019</i>          |
|  |                                 | <b>Less than once a month</b> |              | <b>Once a month or more</b> |                 | <b>Unadjusted odds ratio</b> | <b>95% CI</b> | <b><i>P</i> value</b> |
| <b>Location of meal consumption:</b>           | Outside the home                | 334/570 (58.6)                |              | 75/148 (50.7)               |                 | 0.611                        | 0.418-0.984   | <i>0.01</i>           |
| Italics denote significance at <i>P</i> <0.05. |                                 |                               |              |                             |                 |                              |               |                       |

**Serostatus and Risk Factor Study, 2015-2016.**



**Table 4. Adjusted Associations (Adjusted Odds Ratios) of *Toxoplasma gondii* Seropositivity and Age and Behavioural Risk and Protective Factors by Sex of Old Order Amish Participants, *Toxoplasma gondii* Serostatus and Risk Factor Study, 2015-2016**

| Factor   |   | Male                |                   |               | Female              |                       |                   |
|--|---|---------------------|-------------------|---------------|---------------------|-----------------------|-------------------|
|  |   | Adjusted odds ratio | 95% CI            | P value       | Adjusted odds ratio | 95% CI                | P value           |
| <b>Age</b>   | Female  |                     |                   |               | 1.027               | 1.016 –<br>–<br>1.038 | <i>&lt;0.0001</i> |
|  | Male  | 1.015               | 1.001 –<br>1.030  | <i>0.0348</i> |                     |                       |                   |
| <b>Pets</b>  | Has pets                                      | 0.819               | 0.442 –<br>1.520  | 0.5273        | 1.048               | 0.673 –<br>–<br>1.632 | 0.8364            |
|  | Has kittens                                   | 1.408               | 0.882 –<br>2.247  | 0.1511        | 0.917               | 0.617 –<br>–<br>1.363 | 0.6684            |
|  | Has cats                                      | 1.112               | 0.704 –<br>1.758  | 0.6481        | 1.278               | 0.892 –<br>–<br>1.830 | 0.1814            |
|  | Cleaned the litterbox                         | 5.241               | 1.165 –<br>23.584 | <i>0.0309</i> | 1.466               | 0.578 –<br>–<br>3.723 | 0.4205            |
| <b>Meat consumption*</b>   | Ate rare meat                                 | 1.765               | 0.901 –<br>3.458  | 0.0978        | 1.45                | 0.762 –<br>–<br>2.760 | 0.2579            |
|  | Ate raw meat                                  | 1.111               | 0.446 –<br>2.77   | 0.8216        | 3.536               | 1.469 –<br>–<br>8.510 | <i>0.0048</i>     |
|  | Ate rare ground beef                          | 2.988               | 0.640 –<br>13.96  | 0.164         | 2.327               | 0.687 –<br>–<br>7.889 | 0.175             |
| <b>Unpasteurized milk consumption**</b>  | Consumed unpasteurized cow or goat products** | 1.481               | 0.719–<br>3.050   | 0.2872        | 1.863               | 1.086–<br>3.197       | <i>0.0239</i>     |
|  | Consumed unpasteurized cow milk products      | 1.450               | 0.719–<br>2.926   | 0.2996        | 1.930               | 1.127–<br>3.304       | <i>0.0166</i>     |
| <b>Washing hands</b>   | Always washing hands after touching raw meat  | 0.931               | 0.519 –<br>1.672  | 0.8115        | 0.462               | 0.26 –<br>0.823       | <i>0.0087</i>     |
| <p>Italics denote significance at <math>P &lt; 0.05</math>.<br/> *Only 1 respondent indicated raw ground pork intake; results, therefore, not shown.<br/> **Only 2 respondents indicated consumption of unpasteurized goat milk products; 421 indicated intake of unpasteurized cow milk products.</p> |   |                     |                   |               |                     |                       |                   |