

## Male-to-Female Presumed Transmission of Toxoplasmosis Between Sexual Partners

Jana Hlaváčová, Jaroslav Flegr, Karel Řežábek, Pavel Calda, and Šárka Kaňková

Correspondence to Jana Hlaváčová, Department of Philosophy and History of Science, Faculty of Science, Charles University, Viničná 7, CZ – 128 44 Prague, Czech Republic, Tel: +420721622339  
(e-mail: [hlava.jan@natur.cuni.cz](mailto:hlava.jan@natur.cuni.cz))

Author affiliations: Department of Philosophy and History of Science, Faculty of Science, Charles University, Viničná 7, CZ – 128 44 Prague, Czech Republic (Jana Hlaváčová, Jaroslav Flegr, and Šárka Kaňková); Center for Assisted Reproduction, Department of Obstetrics and Gynaecology, First Faculty of Medicine, Charles University and General University Hospital, Apolinářská 18, CZ – 128 08 Prague, Czech Republic (Karel Řežábek); Fetal Medicine Center, Department of Obstetrics and Gynaecology, First Faculty of Medicine, Charles University and General University Hospital, Apolinářská 18, CZ – 128 08 Prague, Czech Republic (Pavel Calda).

Funding: This research was supported by Charles University Grant Agency (Grant 104218); Czech Science Foundation Grant (Grant 18-13692S); Charles University Research Center Program (Grant 204056); and the Ministry of Health of the Czech Republic (Grant RVO-VFN64165).

© The Author(s) 2020. Published by Oxford University Press on behalf of the Johns Hopkins Bloomberg School of Public Health. All rights reserved. For permissions, please e-mail: [journals.permissions@oup.com](mailto:journals.permissions@oup.com).

Conflict of interest: None declared.

Running head: Sexual Transmission of Toxoplasmosis

Acknowledgments: We would like to thank Anna Pilátová, Ph.D. for final revisions of our text, and Petr Tureček, Ph.D. for advice on the use of statistical methods.

## ABSTRACT

Toxoplasmosis is one of the most widespread human parasitoses in developed countries. Sexual transmission has been confirmed in several animal species, and indirect evidence suggests that it may occur in humans. We compared the seropositivity to *Toxoplasma gondii* in couples who visited the Center for Assisted Reproduction in Prague from June 2016 to June 2018 and analyzed various risk factors including the serological status of sexual partner. By comparing the risk factors in men and women, we tested the hypothesis of man-to-woman sexual transmission of toxoplasmosis. The prevalence of toxoplasmosis in women with infected male partners (25.6%;  $n = 156$ ) was higher than in women with uninfected male partners (18.2%;  $n = 477$ ;  $P = 0.045$ ). Therefore, partner's seropositivity seems to be a risk factor for infection in women ( $n = 593$ ; prevalence ratio = 1.418;  $P = 0.045$ ) but not in men ( $n = 573$ ; prevalence ratio = 1.058;  $P = 0.816$ ). Our results support the hypothesis of the sexual transmission of *Toxoplasma* from men to women. The risk may seem relatively low, but transmission can occur during unprotected sexual intercourse, which may be at the time of conception. Due to the risk of congenital toxoplasmosis, a lower risk of infection than that observed in our study can represent a serious health problem.

**Keywords.** *Toxoplasma*, sexually transmitted diseases, risk factors, semen

The prevalence of toxoplasmosis in human populations ranges mainly from 20 to 80% depending on age, cultural habits and environmental factors. For example, in the 1990s, seroprevalence in women of childbearing age ranged from 37% to 58% in Central European countries, Poland, Croatia, Slovenia, Australia, and northern Africa. Seroprevalence was higher in several Latin American (51–72%) and West African (54–77%) countries. Lower seroprevalence has been reported in Southeast Asia, China, and Korea, as well as in countries with cold climate, such as Scandinavian ones (4–39%) (1). Definitive hosts of *Toxoplasma gondii* are representatives of any feline species and its intermediate hosts can be any warm-blooded vertebrates. In the definitive host, oocysts form in the small intestine and are released into the environment along with feces (2). In humans, there are two clinically distinct phases of postnatally acquired toxoplasmosis. During the acute phase, characterized by symptoms similar to viral or bacterial infection, tachyzoites rapidly multiply in various cells of the host body. In immunocompetent individuals, this phase spontaneously passes into a latent stage, during which bradyzoites slowly multiply in tissue cysts consisting of transformed infected host cells (1). Tissue cysts can occur in the central nervous system, skeletal and cardiac muscles, lungs, liver, kidneys, or reproductive organs (3, 4). In infected individuals, the infection persists for the rest of their life.

A serious form of this disease is prenatally acquired congenital toxoplasmosis, whose prevalence ranges from 0.1 to 3 per 1,000 pregnancies (5). Placental and fetal infections can occur in women infected shortly before or during pregnancy (6). In some cases, this infection leads to a stillbirth or serious neurological impairment of the child (7, 8). Some infections may initially be asymptomatic in newborns but later in life lead to slower mental development, blindness, or epilepsy (7). The probability of vertical transmission increases from 6% in the first trimester to 72% in the third trimester. However, infection in the first trimester has the most serious clinical impact on the fetus (9). Possible sources of postnatally acquired infection are food or water contaminated with oocysts from cat's excrement or consumption of tissue cysts in raw or undercooked meat of intermediate hosts (1).

Sexual transmission of toxoplasmosis has been observed in several animal species. In rats and sheep who mated with an infected male, both females and their offspring were infected (10, 11). *Toxoplasma* has sexually transmitted also during the natural mating of goats (12). Female dogs,

rabbits, and sheep were infected after artificial insemination with infected semen (13–15). The parasite's DNA, cysts, or tachyzoites were found in the reproductive organs of most of the abovementioned animal species (10, 13, 16, 17). *Toxoplasma* was also found in the semen and reproductive organs of experimentally infected male pigs (18) and cattle (19).

It has been found that there is a difference in response to *Toxoplasma* infection between female and male mice. Male mice rapidly respond to infection with high levels of TNF- $\alpha$  and IFN- $\gamma$ , which helps regulate parasite proliferation. Females do not respond as quickly as males and suffer therefore from a lower survival rate as well as higher *Toxoplasma* cyst burden (20).

A review of parasitological literature shows that a large proportion of *Toxoplasma* infections in humans cannot be explained by contact with any known risk factor (21). For instance, in 52% of women who gave birth to children with congenital toxoplasmosis, professionals were unable to find any exposure to a known risk factor (22). Some scientists, therefore, propose the existence of unknown risk factors, including men-to-women transmission of toxoplasmosis via sexual intercourse (23) and oral sex (24). So far, this hypothesis is supported only by indirect evidence. The prevalence of toxoplasmosis in women of childbearing age correlates with the prevalence of some sexually transmitted diseases in a large set of WHO-member countries (23). Unprotected sexual intercourse can be a shared risk factor. The probability of *Toxoplasma* infection in pregnant women positively correlates with the amount of unprotected sex before pregnancy. In infected women, the amount of unprotected sex with their child's father even correlated positively with the concentration of antibodies against *Toxoplasma*. It suggests that especially women with acute and post-acute infections have a recent history of unprotected sex (23). Moreover, research even found an association between *Toxoplasma* infection and the number of reported sexual partners in subjects with psychiatric diagnoses (25). A high prevalence of toxoplasmosis has been reported in men who have sex with men (26) and sex workers (27). Unprotected sex can result in a *Toxoplasma* infection but also lead to conception and congenital toxoplasmosis in a child. Indirect support for hypothesis comes from the USA, where a higher prevalence of toxoplasmosis was observed in fathers of congenitally infected children (36%) than in the general male population (9.8%). Moreover, 13% of fathers tested up to 1 year after the child's birth had high levels of antibodies, which indicates recently acquired infection

(28). An old study demonstrated the presence of *Toxoplasma* zoites in human ejaculate (29), but since then no other study has confirmed this. Overall, the published results of animal and human studies provide strong support for the hypothesis of sexual transmission in humans.

The aim of this cross-sectional study was to find direct epidemiological evidence for the sexual transmission of toxoplasmosis by semen. For this purpose, we examined the population of couples for toxoplasmosis and computed prevalence ratios for various risk factors, including partner's seropositivity, separately in female and male populations. We studied whether the transmission of toxoplasmosis in couples is unidirectional, i.e. whether a man's seropositivity is an epidemiological risk factor for his female partner, while a woman's seropositivity is not a risk factor for her male partner.

## METHODS

### Study design and participants

Data collection was conducted in the form of a cross-sectional study that took place from June 2016 to June 2018 in collaboration with the Center for Assisted Reproduction at the Gynaecological and Obstetric Clinic of the First Faculty of Medicine of the Charles University and the General University Hospital in Prague. The study initially involved 749 couples who visited the Center for Assisted Reproduction with fertility problems. Informed consent was obtained from all participants. Blood samples for serological testing for toxoplasmosis were taken during a routine examination.

This research has been approved by Ethics Committee of General University Hospital in Prague (No. 384/16; 92/17) and by Institutional review board of Faculty of Science, Charles University (No. 2015/29).

### Questionnaire

Both partners separately completed a questionnaire containing questions on age, relationship with the partner, medical history, and epidemiological risk factors. The questionnaire also included the following questions. "Have you been exposed to the risk of sexually transmitted diseases (sex without condoms)? 1) never; 2) minimally (with 1-2 people over lifetime); 3) rarely (with 3-5 people over

lifetime); 4) sometimes yes (with 6-10 people over lifetime); 5) very often (with more than 10 people over lifetime)”; “Population of the town or village where you spent most of your childhood: 1) up to 1,000; 2) 1-5,000; 3) 5-50,000; 4) 50-100,000; 5) 100-500,000; 6) over 500,000“; “Cat as a household pet: 1) never in our family; 2) in the past and only briefly; 3) only in the past but for many years; 4) currently one cat; 5) currently two cats; 6) currently three cats; 7) currently more than three cats“; “Have you eaten poorly washed root vegetables (radishes, carrots...)?”; “Have you eaten or tasted raw meat?”; “Have you been in physical contact with garden soil without using gloves?”. The answers to the last three questions were: “1) never; 2) minimally (1-2 times over lifetime); 3) rarely (3-5 times over lifetime); 4) occasionally (6-15 times over lifetime); 5) very often (16-50 times over lifetime); 6) more than 50 times over lifetime”. The “cat” category was recorded as an ordinal variable “number of cats kept as pets” (1,2,3=0; 4=1; 5=2; 6,7=3).

#### Serological testing for toxoplasmosis

Testing for toxoplasmosis was performed in the National Reference Laboratory for Toxoplasmosis at the State Health Institute in Prague by complement fixation test and by the ELISA IgG test (TestLine Clinical Diagnostics). A negative result of both tests indicated the person was not infected with *Toxoplasma gondii*, while positive results of both tests indicated the presence of anamnestic anti-*Toxoplasma* antibodies. In the case of high levels of IgG antibodies, IgM and IgA antibodies assays were also performed. The presence of high levels of IgM and IgA antibodies indicates acute toxoplasmosis, a recent infection.

#### Statistical analysis

One couple was excluded from the analysis due to suspected acute toxoplasmosis in man. Another 64 couples with inconclusive test results (the result of one test was positive, the other negative) were also excluded from the analysis. Due to a possibility of *Toxoplasma* infection from previous partners, we excluded, prior to the analysis of men-to-women transmission, 51 couples in which a *Toxoplasma*-infected women reported exposure to the risk of sexually transmitted diseases (sex without condoms) with more than two people over their lifetime or did not answer the question.

Similarly, before the second analysis of women-to-men transmission, we excluded 69 couples in which a *Toxoplasma*-infected men reported exposure to the risk of sexually transmitted diseases (sex without condoms) with more than two people over their lifetime or did not answer the question.

The data were analyzed in a statistical program R, version 3.6.3 (30). The Wilcoxon test was used to compare the age in particular groups. Contingency tables and logistic regression methods were used to search for concordance in *Toxoplasma*-seropositivity status of partners. In the statistical model, the dependent variable was woman's/man's seropositivity, while independent variables were partner's seropositivity, age of woman/man, size of town of residence in childhood, number of cats, ingestion of poorly washed root vegetables, ingestion of raw meat, and contact with garden soil. The probability of *Toxoplasma* infection increases with subject's age, which is why we also tested the association between seropositivity of both partners by a partial Kendall correlation with a woman's age controlled. We used the "prLogistic" package to calculate prevalence ratios (31) and the "mice" package to calculate missing values by multiple imputation method (32). Missing values were calculated if the woman/man did not answer one question. If more answers were missing (most answers related to risk factors), the participant was excluded (1.42% of women and 3.25% of men).

All data are available in online open-access repository Figshare (doi:10.6084/m9.figshare.9198008).

## RESULTS

### Descriptive statistics

The dataset contained 684 couples. The proportion of infected women was not significantly higher than the proportion of infected men (26% vs. 24.1%;  $\chi^2 = 0.658$ ;  $P = 0.417$ ). The mean length of the relationship was app. 6.7 years (min. 4 months; max. 24 years).

The first analysis (assessing man-to-woman sexual transmission of toxoplasmosis) was performed on 633 couples. Women's mean age (33.2; SD = 4.7) was lower than the mean age of their male partners (35.7; SD = 5.4;  $P < 0.001$ ). The mean age of infected women (33.7; SD = 4.3) did not differ from the mean age of uninfected women (33; SD = 4.8;  $P = 0.119$ ) and the mean age of infected

male partners (36.1; SD = 5.4) did not differ from the mean age of uninfected male partners (35.6; SD = 5.4;  $P = 0.197$ ).

The second analysis, which tested woman-to-man sexual transmission of toxoplasmosis, was performed on 615 couples. Men's mean age (35.6; SD = 5.4) was higher than the mean age of their female partners (33.2; SD = 4.8;  $P < 0.001$ ). The mean age of infected men (35.7; SD = 5.1) did not differ from the mean age of uninfected men (35.5; SD = 5.4;  $P = 0.651$ ), but the mean age of infected female partners (33.9; SD = 4.4) was significantly higher than the mean age of uninfected female partners (32.9; SD = 4.9;  $P = 0.025$ ). Further details about the population structure are shown in Table 1.

#### Study of man-to-woman sexual transmission of toxoplasmosis

Prevalence of toxoplasmosis in women with infected male partners (25.6%;  $n = 156$ ) was compared to toxoplasmosis prevalence in women with uninfected male partners (18.2%;  $n = 477$ ) using contingency tables and Pearson's chi-squared test. The difference in prevalence between these two groups of women was significant ( $\chi^2 = 4.016$ ;  $df = 1$ ;  $P = 0.045$ ). Women with infected male partners thus are at a higher risk of toxoplasmosis than those with uninfected male partners (risk ratio [RR] = 1.406; 95% confidence interval: 1.013, 1.951). Also, the robust non-parametric partial Kendall correlation with woman's age controlled showed a positive association between seropositivity of both partners (partial Tau = 0.079;  $P = 0.003$ ).

Logistic regression showed a statistically significant association between women's seropositivity and the seropositivity of their male partners, as well as the size of place of residence in childhood. Other potential factors we considered, i.e. woman's age, number of cats, ingestion of poorly washed root vegetables, ingestion of raw meat, and contact with garden soil, did not correlate with the women's seropositivity. A total of 40 women did not answer all questions about risk factors in the questionnaire and as a result, only 593 women were included in logistic regression analysis. However, the results were similar even after calculating the missing values using the multiple imputation method ( $n = 624$ ) (Table 2).



## Study of woman-to-man sexual transmission of toxoplasmosis

We compared the prevalence of toxoplasmosis in men with infected ( $n = 156$ ; prevalence = 17.3%) and uninfected ( $n = 459$ ; prevalence = 15%) female partners using contingency tables and Pearson's chi-squared test. We found no significant difference in prevalence between the two groups ( $\chi^2 = 0.457$ ;  $df = 1$ ;  $P = 0.499$ ). The risk ratio of 1.151 (95% confidence interval: 0.767, 1.728) indicated no increase in contagion risk for men with infected female partners. Similarly, partial Kendall correlation with man's age controlled did not reveal an association between seropositivity of both partners (partial Tau = 0.027;  $P = 0.318$ ).

Logistic regression likewise revealed no statistically significant association between men's seropositivity and the seropositivity of their female partners. We did, however, identify two factors, namely the size of place of residence in childhood and contact with garden soil, which correlated with the seropositivity of men. A total of 42 men did not answer all questions about the risk factors and as a result, 573 men were included in this analysis. Again, the results were similar after calculating the missing values by the multiple imputation method ( $n = 595$ ) (Table 3).

## DISCUSSION

Our results suggest that some women could get infected with *Toxoplasma* from their male partners. There was a 1.4 times higher prevalence of toxoplasmosis in women in couples with infected male partners than in couples with uninfected male partners (25.6% vs. 18.2%). For women, male partner's infection is a risk factor while for men, infection of their female partner is not. These results support the hypothesis of unidirectional *Toxoplasma* transmission between sexual partners and represent the first indirect evidence for transmission by semen in humans.

A recent epidemiological study performed in the Czech Republic reported higher toxoplasmosis prevalence in women (34.5%) than in men (24%) (33). This study had also shown that until the age of 19, the prevalence in males and females is similar. Around 30 years of age, the prevalence is significantly higher in women than in men. After this age, the prevalence in men stagnates or decreases, while in women it increases up to 50 years of age. All this evidence is fully

compatible with the proposed existence of a unique (sexual) transmission route of toxoplasmosis for women, which starts to have an effect after the commencement of regular sexual activity.

Women infected during unprotected sexual intercourse could thus be responsible for the reported increase of prevalence of toxoplasmosis. On the other hand, however, when we looked at our entire dataset including *Toxoplasma*-infected subjects who reported exposure to the risk of sexually transmitted diseases with more than two people over lifetime, we did not see a significantly higher proportion of infected women (26%) than infected men (24.1%). This could be due to our atypical sample (subfertile individuals). A higher prevalence of toxoplasmosis was observed in subfertile men (34) and subfertile women (35). The traditional explanation for increasing prevalence of toxoplasmosis in women after the age of 20 is based on their engagement in cooking rather than regular sexual activity (23, 35). Nevertheless, our study did not identify raw meat consumption as a risk factor for infection, and the absence of a positive association between toxoplasmosis and raw meat consumption makes this traditional explanation less probable.

Sexual transmission of toxoplasmosis has been confirmed in many animal species and is currently considered in humans (23, 27). Direct testing of sexual transmission in humans is impossible and only indirect evidence is therefore available to support the hypothesis of sexual transmission of toxoplasmosis in humans. Recently, a high prevalence of toxoplasmosis was observed in fathers of congenitally infected children. More than 13% of fathers tested up to one year after the child's birth had a high concentration of anti-*Toxoplasma* IgG and were thus probably infected recently (28). This also suggests that sexual transmission of toxoplasmosis may occur during the acute or early post-acute phase of the zoonosis. In future studies, it would be crucial to find out which form of *Toxoplasma* (tachyzoites or bradyzoites) is present in the ejaculate of infected men in the latent resp. the acute phase of infection.

One could argue that repeated contact with a shared source of infection resulting from sharing residence, food, and eating habits could also play a role in the partner's (co)infection. Such shared exposure to the usual risk factors cannot, however, explain why male infection increases the risk of female infection, while female infection does not increase the risk of male infection. This pattern can be expected in the transmission of toxoplasmosis by ejaculate and no other explanation readily comes

to mind. Our data thus seem to support the hypothesis of male-to-female transmission of toxoplasmosis rather than the hypothesis based on common risk factors.

#### Limitations

Our atypical data sample may seem to be a limitation when it comes to comparing the prevalence of toxoplasmosis in general. At a second glance, however, the subfertile individuals are an ideal sample for testing the hypothesis of sexual transmission because it involves couples who had been trying to conceive for a long time and therefore had frequent unprotected sex.

We tried to eliminate or statistically control potential confounding variables (e.g. exclusion of subjects who had been exposed to the risk of sexually transmitted diseases with more than two people over lifetime because of the possibility of *Toxoplasma* infection from previous partners). Nevertheless, the toxoplasmosis status of partners before the formation of the current couple was beyond our control. Nevertheless, it should be emphasized that the existence of such confounding variables could increase the risk of false-negative test results (non-detection of existing effects), not false-positive test results (detection of non-existent effects).

The observed effect size for sexual transmission was low compared to the risk posed by other factors such as contact with garden soil or the consumption of raw meat found in other studies. Again, the small effect size (or small datasets) increases the risk of false-negative but not false-positive results.

A cross-sectional study is inherently observational. Direct evidence for the existence of *Toxoplasma* transmission by semen could only be obtained by a manipulative study, i.e. by experimental infections. Such studies, naturally, cannot be performed on humans.

#### Conclusion

Toxoplasmosis is not officially classified as a sexually transmitted disease. Our results, however, show that men's infection increases the risk of female partner's infection approximately 1.4 times. Sexual transmission of toxoplasmosis, though rare, could have a serious impact on the individuals thus affected and thereby also a general impact on public health. The infection could occur

around the time of conception and result in congenital toxoplasmosis, the most serious form of the disease, and potential fetal damage.

## REFERENCES

1. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol.* 2000;30(12–13):1217–1258.
2. Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin. Microbiol. Rev.* 1998;11(2):267–299.
3. Dubey JP. Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *T. gondii* oocysts and effect of freezing on viability of tissue cysts in pork. *Am. J. Vet. Res.* 1988;49(6):910–913.
4. Martinez-Garcia F, Regadera J, Mayer R, et al. Protozoan infections in the male genital tract. *J Urol.* 1996;156:340–9.
5. Varella IS, Canti ICT, Santos BR, et al. Prevalence of acute toxoplasmosis infection among 41,112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. *Mem. Inst. Oswaldo Cruz.* 2009;104(2):383–388.
6. Robert-Gangneux F, Murat J-B, Fricker-Hidalgo H, et al. The placenta: a main role in congenital toxoplasmosis? *Trends Parasitol.* 2011;27(12):530–536.
7. Lopez A, Dietz VJ, Wilson M, et al. Preventing congenital toxoplasmosis. *MMWR Recomm. Rep.* 2000;49(RR-2):59–68.
8. Wolf A, Cowen D, Paige BH. Toxoplasmic encephalomyelitis: III. A new case of granulomatous encephalomyelitis due to a protozoan. *Am. J. Pathol.* 1939;15(6):657–694.11.
9. Dunn D, Wallon M, Peyron F, et al. Mother-to-child transmission of toxoplasmosis: Risk estimates for clinical counselling. *The Lancet.* 1999;353(9167):1829–33.
10. Dass SAH, Vasudevan A, Dutta D, et al. Protozoan parasite *Toxoplasma gondii* manipulates mate choice in rats by enhancing attractiveness of males. *PLoS One.* 2011;6(11):e27229.
11. Lopes WDZ, Rodriguez JD, Souza FA, et al. Sexual transmission of *Toxoplasma gondii* in sheep. *Vet. Parasitol.* 2013;195(1–2):47–56.
12. Santana LF, Rossi GAM, Gaspar RC, et al. Evidence of sexual transmission of *Toxoplasma gondii* in goats. *Small Rumin. Res.* 2013;115(1–3):130–133.
13. Arantes TP, Lopes WDZ, Ferreira RM, et al. *Toxoplasma gondii*: Evidence for the transmission by semen in dogs. *Exp. Parasitol.* 2009;123(2):190–194.
14. de Moraes ÉPBX, Batista AM, Faria EB, et al. Experimental infection by *Toxoplasma gondii* using contaminated semen containing different doses of tachyzoites in sheep. *Vet. Parasitol.* 2010;170(3–4):318–322.

15. Liu S, Qin C, Yao Z, et al. Study on the transmission of *Toxoplasma gondii* by semen in rabbits. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*. 2006;24(3):166–170.
16. Liu SG, Zhang HZ, Li X, et al. Dynamic observation of polypide in semen and blood of rabbits infected with *Toxoplasma* tachyzoites. *Chin. Med. J*. 2006;119(8):701–704.
17. Santana LF, da Costa AJ, Pieroni J, et al. Detection of *Toxoplasma gondii* in the reproductive system of male goats. *Rev. Bras. Parasitol. Veterinária*. 2010;19(3):179–182.
18. Moura AB, Costa AJ, Jordao Filho S, et al. *Toxoplasma gondii* in semen of experimentally infected swine. *Pesq. Vet. Bras*. 2007;27(10):430–434.
19. Scarpelli L, Lopes WDZ, Migani M, et al. *Toxoplasma gondii* in experimentally infected *Bos taurus* and *Bos indicus* semen and tissues. *Pesq. Vet. Bras*. 2009;29(1):59–64.
20. Roberts CW, Cruickshank SM, Alexander J. Sex-determined resistance to *Toxoplasma gondii* is associated with temporal differences in cytokine production. *Infect. Immun*. 1995;63(7):2549–2555.
21. Petersen E, Vesco G, Villari S, et al. What do we know about risk factors for infection in humans with *Toxoplasma gondii* and how can we prevent infections? *Zoonoses Public Health*. 2010;57(1):8–17.
22. Boyer KM, Holfels E, Roizen N, et al. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. *Am. J. Obstet. Gynecol*. 2005;192(2):564–571.
23. Flegr J, Klapilová K, Kaňková Š. Toxoplasmosis can be a sexually transmitted infection with serious clinical consequences. Not all routes of infection are created equal. *Med. Hypotheses*. 2014;83(3):286–289.
24. Kaňková Š, Hlaváčová J, Flegr J. Oral sex: A new, and possibly the most dangerous, route of toxoplasmosis transmission. *Med. Hypotheses*. 2020;141:109725.
25. Alvarado-Esquivel C, Alanis-Quiñones O-P, Arreola-Valenzuela M-Á, et al. Seroepidemiology of *Toxoplasma gondii* infection in psychiatric inpatients in a northern Mexican city. *BMC Infect. Dis*. 2006;6:178.
26. Prasetyo AA, Ariapramuda R, Kindi EA, et al. Men having sex with men in Surakarta, Indonesia: demographics, behavioral characteristics and prevalence of blood borne pathogens. *Southeast Asian J. Trop. Med. Public Health*. 2014;45(5):1032–1047.
27. Alvarado-Esquivel C, Sánchez-Anguiano LF, Hernández-Tinoco J, et al. High seroprevalence of *Toxoplasma gondii* infection in female sex workers: a case-control study. *Eur. J. Microbiol. Immunol*. 2015;5(4):285–292.
28. Contopoulos-Ioannidis D, Wheeler KM, Ramirez R, et al. Clustering of *Toxoplasma gondii* infections within families of congenitally infected infants. *Clin. Infect. Dis*. 2015;61(12):1815–1824.
29. Disko R, Braveny I, Vogel P. Untersuchungen zum Vorkommen von *Toxoplasma gondii* im menschlichen Ejakulat. *Z. Tropenmed. Parasitol*. 1971;22:391–397.
30. R Core Team. R: A language and environment for statistical computing. *R Found. Stat. Comput. Vienna Austria*. 2020.

31. Ospina R, Amorim LD. prLogistic: Estimation of Prevalence Ratios using Logistic Models. 2013.
32. Buuren S van, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. *J. Stat. Softw.* 2011;45(1):1–67.
33. Flegr J. Predictors of *Toxoplasma gondii* infection in Czech and Slovak populations: the possible role of cat-related injuries and risky sexual behavior in the parasite transmission. *Epidemiol. Infect.* 2017;145(7):1351–1362.
34. Qi R, Su X, Gao X, et al. *Toxoplasma* infection in males with sterility in Shenyang, China. *Zhonghua Nan Ke Xue = Natl. J. Androl.* 2005;11(7):503–504.
35. El-Tantawy N, Taman A, Shalaby H. Toxoplasmosis and female infertility: is there a correlation? *Am. J. Epidemiol. Infect. Dis.* 2014;2(1):29–32.
36. Kodym P, Malý M, Švandová, E., et al. *Toxoplasma* in the Czech Republic 1923-1999: first case to widespread outbreak. *Int. J. Parasitol.* 2000;(31):125–132.

ORIGINAL UNEDITED MANUSCRIPT

**Table 1.** Distribution of Answers to Selected Questionnaire Questions in *Toxoplasma*-positive and *Toxoplasma*-negative Men and Women, 2016 – 2018

Risk factors	Respondents' answers													
	1		2		3		4		5		6		7	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Childhood residence TPW <sup>a</sup>	27	22.9	18	15.3	34	28.8	11	9.3	8	6.8	20	17		
Childhood residence TNW <sup>b</sup>	57	11.6	71	14.4	148	30	51	10.3	31	6.3	135	27.4		
Childhood residence TPM <sup>c</sup>	28	29.8	15	16	20	21.3	11	11.7	4	4.3	16	17		
Childhood residence TNM <sup>d</sup>	70	13.8	58	11.4	160	31.6	41	8.1	31	6.1	147	29		
Household cats TPW <sup>a</sup>	51	40.5	16	12.7	23	18.3	24	19.1	11	8.7	0	0	1	0.8
Household cats TNW <sup>b</sup>	189	38.2	73	14.8	84	17	100	20.2	32	6.5	8	1.6	9	1.8
Household cats TPM <sup>c</sup>	31	32.3	17	17.7	20	20.8	18	18.8	5	5.2	3	3.1	2	2.1
Household cats TNM <sup>d</sup>	204	39.8	90	17.6	84	16.4	91	17.8	35	6.8	2	0.4	6	1.2
Root vegetables TPW <sup>a</sup>	21	16.9	28	22.6	19	15.3	28	22.6	14	11.3	14	11.3		
Root vegetables TNW <sup>b</sup>	49	9.8	80	16	109	21.8	146	29.2	58	11.6	58	11.6		
Root vegetables TPM <sup>c</sup>	6	6.5	12	12.9	10	10.8	33	35.5	18	19.4	14	15.1		
Root vegetables TNM <sup>d</sup>	38	7.6	60	12	74	14.8	135	27	77	15.4	116	23.2		
Raw meat TPW <sup>a</sup>	18	14.6	24	19.5	26	21.1	36	29.3	14	11.4	5	4.1		
Raw meat TNW <sup>b</sup>	81	16.2	82	16.4	86	17.2	151	30.1	73	14.6	28	5.6		
Raw meat TPM <sup>c</sup>	8	8.7	18	19.6	12	13	34	37	15	16.3	5	5.4		
Raw meat TNM <sup>d</sup>	55	10.9	63	12.5	65	12.9	160	31.8	97	19.3	64	12.7		
Garden soil TPW <sup>a</sup>	4	3.3	6	4.9	11	9	31	25.4	32	26.2	38	31.2		
Garden soil TNW <sup>b</sup>	13	2.6	14	2.8	50	10	126	25.2	111	22.2	186	37.2		
Garden soil TPM <sup>c</sup>	1	1.1	2	2.2	2	2.2	11	11.8	28	30.1	49	52.7		
Garden soil TNM <sup>d</sup>	13	2.6	13	2.6	36	7.2	93	18.5	110	21.9	237	47.2		

Full details of questions and respondents' answers are described in Methods – Questionnaire.

<sup>a</sup>*Toxoplasma*-positive women

<sup>b</sup>*Toxoplasma*-negative women

<sup>c</sup>*Toxoplasma*-positive men

<sup>d</sup>*Toxoplasma*-negative men

**Table 2.** Association Between Various Factors for *Toxoplasma* Infection and Women's Seropositivity, 2016 – 2018

Risk factors	PR <sup>a</sup>	95% CI <sup>b</sup>	P <sup>c</sup>	P <sup>d</sup>
Partner's infection	1.418	0.981, 2.049	0.045	0.063
Woman's age	1.033	0.987, 1.081	0.072	0.056
Size of place of residence in childhood	0.836	0.748, 0.933	<0.001	<0.001
Number of cats kept as pets	0.842	0.656, 1.081	0.163	0.296
Ingestion of poorly washed root vegetables	0.900	0.792, 1.024	0.100	0.119
Ingestion of raw meat	0.978	0.869, 1.101	0.713	0.970
Contact with garden soil	0.957	0.831, 1.101	0.545	0.600

This table shows the results of logistic regression.

<sup>a</sup>prevalence ratio

<sup>b</sup>confidence interval

<sup>c</sup>p-value

<sup>d</sup>p-value after multiple imputation method

ORIGINAL UNEDITED MANUSCRIPT



**Table 3.** Association Between Various Factors for *Toxoplasma* Infection and Men's Seropositivity, 2016 – 2018

Risk factors	PR <sup>a</sup>	95% CI <sup>b</sup>	P <sup>c</sup>	P <sup>d</sup>
Partner's infection	1.058	0.657, 1.704	0.816	0.984
Man's age	1.015	0.974, 1.057	0.470	0.464
Size of place of residence in childhood	0.831	0.732, 0.943	0.004	0.002
Number of cats kept as pets	1.108	0.825, 1.471	0.512	0.358
Ingestion of poorly washed root vegetables	0.901	0.768, 1.059	0.204	0.201
Ingestion of raw meat	0.914	0.786, 1.064	0.248	0.126
Contact with garden soil	1.307	1.020, 1.676	0.019	0.013

This table shows the results of logistic regression.

<sup>a</sup>prevalence ratio

<sup>b</sup>confidence interval

<sup>c</sup>p-value

<sup>d</sup>p-value after multiple imputation method

ORIGINAL UNEDITED MANUSCRIPT