Original Contribution

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

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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 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 male-to-female 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, a 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 *T. gondii* 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. Because of the risk of congenital toxoplasmosis, a lower risk of infection than that observed in our study can represent a serious health problem.

risk factors; semen; sexually transmitted diseases; *Toxoplasma*

Abbreviations: Ig, immunoglobulin; SD, standard deviation.

The prevalence of toxoplasmosis in human populations generally ranges 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 in the Scandinavian region (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 2 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 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 of their respective species, both females and their offspring were infected (10, 11). *Toxoplasma* has been 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 aforementioned 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).

There is a difference in response to *Toxoplasma* infection between female and male mice. Male mice rapidly respond to infection, with high levels of tumor necrosis factor-α and interferon-γ, which help regulate parasite proliferation. Females do not respond as quickly as males and, therefore, have 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 male-to-female 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 World Health Organization–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*. These findings suggest that especially women with acute and postacute infections have a recent history of unprotected sex (23). Moreover, an association even has been found between *Toxoplasma* infection and the number of reported sexual partners in patients 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 United States, 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 indicate recently acquired infection (28). A 1971 study demonstrated the presence of *Toxoplasma* zoites in human ejaculate (29), but since then, no other study has confirmed this, to our knowledge. 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 epidemiologic 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 epidemiologic risk factor for his female partner, whereas 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 Gynecological 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 collected during a routine examination. This research was approved by the Ethics Committee of General University Hospital in Prague (No. 384/16; 92/17) and by Institutional Review Board of the Faculty of Science, Charles University (No. 2015/29).

Questionnaire

Both partners separately completed a questionnaire containing questions about age, relationship with the partner, medical history, and epidemiologic 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) <1,000; 2) 1,000–4,999; 3) 5,000–49,999; 4) 50,000–99,999; 5) 100,000–499,999; 6) ≥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 1; 5) currently 2 cats; 6) currently 3 cats; 7) currently more than 3 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 answer options to the last 3 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 enzyme-linked immunosorbent assay immunoglobulin (Ig) G test (TestLine Clinical Diagnostics, Brno-Královo Pole, Czech Republic). A negative result of both tests indicated the person was not infected with *T. gondii*, whereas 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 and a recent infection.

Statistical analysis

One couple was excluded from the analysis because of suspected acute toxoplasmosis in the man. Another 64 couples with inconclusive test results (i.e., the result of 1 test was positive, the other negative) were also excluded from the analysis. Because of a possibility of *Toxoplasma* infection from previous partners, we excluded, prior to the analysis of male-to-female transmission, 51 couples in whom a *Toxoplasma*-infected woman reported exposure to the risk of sexually transmitted diseases (e.g., sex without condoms) with more than 2 people over their lifetime or did not answer the question. Similarly, before the second analysis of female-to-male transmission, we excluded 69 couples in whom a *Toxoplasma*-infected man reported exposure to the risk of sexually transmitted diseases (e.g., sex without condoms) with more than 2 people over their lifetime or did not answer the question.

The data were analyzed in R, version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) (30). The Wilcoxon test was used to compare 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 the participant's seropositivity, and independent variables were partner's seropositivity, age of the participant, 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 one'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 participant did not answer 1 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 the online open-access repository Figshare (doi:10.6084/m9.figshare.9198008).

RESULTS

Descriptive statistics

The data set 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 approximately 6.7 years (minimum,. 4 months; maximum, 24 years).

The first analysis (assessing male-to-female sexual transmission of toxoplasmosis) was conducted with 633 couples. Women's mean age (33.2 years; standard deviation (SD) = 4.7) was lower than the mean age of their male partners (35.7 years; SD = 5.4; $P < 0.001$). The mean age of infected women (33.7 years; SD = 4.3) did not differ from the mean age of uninfected women (33.0 years; SD = 4.8; $P = 0.119$), and the mean age of infected male partners (36.1 years; SD = 5.4) did not differ from the mean age of uninfected male partners (35.6 years; SD = 5.4; $P = 0.197$).

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

Study of male-to-female 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 the Pearson χ^2 test. The difference in prevalence between these 2 groups of women was significant ($\chi^2 = 4.016$; df = 1; $P = 0.045$). Women with infected male partners thus were at a higher risk of toxoplasmosis than those with uninfected male partners (risk ratio = 1.406; 95% confidence interval: 1.013, 1.951). Also, the robust, nonparametric, partial Kendall correlation, with the woman's age controlled for, indicated a positive association between seropositivity of both partners (partial $\tau = 0.079$; $P = 0.003$).

According to logistic regression, there was 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

Table 1. Distribution of Answers to Selected Questionnaire Questions in *Toxoplasma*-Positive and *Toxoplasma*-Negative Men and Women, Prague, Czech Republic, 2016–2018

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

Abbreviations: TNM, *Toxoplasma*-negative men; TNW, *Toxoplasma*-negative women; TPM, *Toxoplasma*-positive men; TPW, *Toxoplasma*-positive women.

analysis. However, the results were similar even after calculating the missing values using the multiple imputation method ($n = 624$) (Table 2).

Study of female-to-male 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 χ^2 test. We found no significant difference in prevalence between the 2 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 the man's age controlled for, 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. However, we did identify 2 factors, namely the size of place of residence in childhood and contact with garden soil, that 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* transmitted from their male partners. There

Table 2.   Logistic Regression Results Showing the Associations of Various Factors for *Toxoplasma* Infection With Women's Seropositivity, Prague, Czech Republic, 2016–2018

Risk Factors	PR	95% CI	P Value	P′ Value[a]
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
No. 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

Abbreviations: CI, confidence interval; PR, prevalence ratio.
[a] *P* value after multiple imputation method.

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, a male partner's infection is a risk factor, whereas for men, infection of their female partner is not. These results support the hypothesis of unidirectional *Toxoplasma* transmission between sexual partners and, to our knowledge, represent the first indirect evidence for transmission by semen in humans.

Researchers who conducted a recent epidemiologic study in the Czech Republic reported higher toxoplasmosis prevalence in women (34.5%) than in men (24%) (33). In the same study, the researchers had also shown that until the age of 19 years, the prevalence of toxoplasmosis in boys and girls is similar. At approximately 30 years of age, the prevalence is significantly higher in women than in men. After this age, the prevalence in men stagnates or decreases, whereas 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 thus could be responsible for the reported increase of prevalence of toxoplasmosis. On the other hand, however, when we looked at our entire data set, including participants infected with *Toxoplasma* who reported exposure to the risk of sexually transmitted diseases with more than 2 people over their 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 (i.e., 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 age 20 years is based on their engagement in cooking rather than regular sexual activity (23, 36). 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

Table 3.   Logistic Regression Results Showing the Associations of Various Factors for *Toxoplasma* Infection With Men's Seropositivity, Prague, Czech Republic, 2016–2018

Risk Factors	PR	95% CI	P Value	P′ Value[a]
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
No. 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

Abbreviations: CI, confidence interval; PR, prevalence ratio.
[a] *P* value after multiple imputation method.

humans is impossible; thus, only indirect evidence is 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 1 year after their child's birth had a high concentration of anti-*Toxoplasma* IgG and, thus, probably were infected recently (28). This finding also suggests that sexual transmission of toxoplasmosis may occur during the acute or early postacute 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 and 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, however, cannot explain why male infection increases the risk of female infection, but 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 the sample 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 participants who had been exposed to the risk of sexually transmitted diseases with >2 people over their lifetime because of the possibility of *Toxoplasma* infection from previous partners). However, 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 (i.e., nondetection of existing effects), not false-positive test results (i.e., detection of nonexistent effects).

The observed effect size for sexual transmission was low compared with the risk posed by other factors such as contact with garden soil or the consumption of raw meat that was found in other studies. Again, the small effect size (or small data sets) increased 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, of course, 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 a 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.

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