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SHORT COMMUNICATION

Women infected with parasite *Toxoplasma* have more sons

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Abstract The boy-to-girl ratio at birth (secondary sex ratio) is around 0.51 in most populations. The sex ratio varies between societies and may be influenced by many factors, such as stress and immunosuppression, age, primiparity, the sex of the preceding siblings and the socioeconomic status of the parents. As parasite infection affects many immunological and physiological parameters of the host, we analyzed the effect of latent toxoplasmosis on sex ratios in humans. Clinical records of 1,803 infants born from 1996 to 2004 contained information regarding the mother's age, concentration of anti-*Toxoplasma* antibodies, previous deliveries and abortions and the sex of the newborn. The results of our retrospective cohort study suggest that the presence of one of the most common parasites (with a worldwide prevalence from 20 to 80%), *Toxoplasma gondii*, can influence the secondary sex ratio in humans. Depending on the antibody concentration, the probability of the birth of a boy can increase up to a value of 0.72, $C.I._{95}$=(0.636, 0.805), which means that for every 260 boys born, 100 girls are born to women with the highest concentration of anti-*Toxoplasma* antibodies. The toxoplasmosis associated with immunosuppression or immunomodulation might be responsible for the enhanced survival of male embryos. In light of the high prevalence of latent toxoplasmosis in most countries, the impact of toxoplasmosis on the human population might be considerable.

Keywords Human sex ratio · Secondary sex ratio · Immunosuppression · Manipulation hypothesis · Trivers–Willard hypothesis

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Introduction

The secondary sex ratio in humans (in this article, expressed as the probability of the birth of a boy) is around 0.51 in most populations (Davis et al. 1998). Within the population, the sex ratio may be influenced by many factors, such as stress and immunosuppression (James 1996), age (Jacobsen et al. 1999), the sex of preceding siblings (Renkonen et al. 1962), paternal endocrine disruption (James 2006) and the socioeconomic status of parents (Chacon-Pugnau and Jaffe 1996). It was repeatedly demonstrated that diseases may influence the endocrine and immune systems, and it is not surprising in this context that they may affect sex ratios (for reviews, see James 1996, 2006). The effects of particular factors studied on large population samples are often significant, but their effect size is rather small. Very strong effects are induced

only by microorganisms, e.g. *Wolbachia* (Eubacteria) and *Octosporea* (Microsporidia), which are transmitted vertically by oocytes (not spermatozoa) in many insects, crustaceans and helminths (Knight 2001; Dunn et al. 2001). Until now, no effects of parasitic infection up on the human sex ratio have been published.

Toxoplasma gondii (Apicomplexa) is one of the most common parasitic protozoan in humans. The prevalence of *Toxoplasma* infection varies mostly from 20 to 80% in different territories (Tenter et al. 2000). The main source of the infection for humans is from the consummation of raw or undercooked meat of an intermediate host (especially pigs, sheep and rabbits) containing tissue cysts and from food or water contaminated with soil containing cat feces with oocysts (Tenter et al. 2000; Beatie 1982). Two clinically different forms of postnatally acquired toxoplasmosis exist, depending on the stage of infection and the host's immunocompetence. One form is acute toxoplasmosis, which is characterized by the presence of tachyzoites in the blood and other tissues. In immunocompetent subjects, acute toxoplasmosis spontaneously turns into the other form, i.e. latent toxoplasmosis. The latter is clinically asymptomatic, but it is usually a life-long infection, characterized by the presence of anti-*Toxoplasma* antibodies in serum and of the *Toxoplasma* bradyzoites in tissue cysts, mainly in the nerve and muscular tissues of infected subjects. *Toxoplasma* is a classical model for the study of the so-called manipulation hypothesis (Hutchinson et al. 1980; Berdoy et al. 1995). The behavioral changes observed in infected animals, e.g. impairment of memory, hyperactivity, decreased ability to discriminate between familiar and novel stimuli and impairment of motor performance and coordination (Webster 1994; Berdoy et al. 2000), and in humans, e.g. decrease of psychomotor performance, decrease of novelty seeking and superego strength (Flegr et al. 1996, 2002; Havlíček et al. 2001), are usually considered to be products of the manipulative activity of the parasite, which is primarily aimed to increase the probability of transmission from the intermediate hosts (usually rodents) to a definitive host (any feline species) by predation.

Toxoplasmosis could also influence the human pregnancy. Women with latent toxoplasmosis had seemingly younger foetuses, based on ultrasonography in the presumed 16th week of pregnancy, and probably also a longer gestation period (Flegr et al. 2005). Indirect evidence suggests that women with toxoplasmosis have relaxed stringency of quality control of embryos. Hostomská et al. (1957) discovered that the prevalence of toxoplasmosis in 94 mothers of children with Down syndrome was 84%. The prevalence in the control population of the same age, as well as in the fathers of the children with Down syndrome, was 32%. The high prevalence of toxoplasmosis among mothers of children with Down syndrome can be most easily explained by their relaxed stringency of quality control that can increase the probability of bringing (*Toxoplasma*-infected) children with developmental defects to full term. The same mechanism, which is probably related to toxoplasmosis-associated immunosuppression, is responsible for higher mortality of male embryos. Therefore, the *Toxoplasma*-infected women are expected to have higher sex ratio than *Toxoplasma*-negative controls.

To test this hypothesis, we compared secondary sex ratios in large samples of *Toxoplasma*-positive and *Toxoplasma*-negative women in three private maternity clinics (tests for toxoplasmosis are not routinely done in toll-free state clinics in Czech Republic) and studied a possible correlation between secondary sex ratio and concentration of specific anti-*Toxoplasma* antibodies in women with latent toxoplasmosis. Women of higher socioeconomic status are overrepresented among clients of private clinics. They could differ from the general population in prevalence of toxoplasmosis and also in the sex ratio of their offspring (Trivers and Willard 1973). Therefore, we included also the variable clinic into the models and analyzed the possible correlation between the expensiveness of clinic and the sex ratio.

Materials and methods

Subjects

Data were collected in three clinics in which every pregnant woman was obligatorily tested for toxoplasmosis using the indirect immunofluorescence test (IIFT; Gest) or complement fixation test (CFT; GynCentrum) during the 16th week of pregnancy. IIFT is a more sensitive method than CFT. Therefore, IIFT titre 1:32 roughly corresponds to CFT titre 1:16. All women who delivered between 1996 and 2004, except two women suspected for acute toxoplasmosis, (with high levels of immunoglobulin M or immunoglobulin A anti-*Toxoplasma* antibodies), were included into our cohort study. Anonymized clinical records contained information on the mother's age, concentration of anti-*Toxoplasma* antibodies, previous deliveries and abortions and the sex of the newborn. All three clinics (GEST Centre of Reproductive Medicine, Prague 6; GEST Centre of Reproductive Medicine, Prague 8; and GynCentrum, Prague 9) were located in the city of Prague, Czech Republic. The overall prevalence of *Toxoplasma* positivity was 18% in GEST Centre of Reproductive Medicine, Prague 6; 25% in GEST Centre of Reproductive Medicine, Prague 8; and 32% in GynCentrum, Prague 9. The demographic data for the city of Prague and the Czech Republic (year 2003) were obtained from the Czech Statistical Office (http://www.czso.cz/csu/edicniplan.nsf/p/4019-04).

Statistical analysis

The sex of a newborn, as well as the mother's age and number of previous pregnancies and abortions, was recorded and used for further analysis. The effects of a mother's age as a continuous predictor, the mother's *Toxoplasma* positivity, the mother's primiparity (if they are primiparous mothers without previous abortions) and the sample (clinic) as categorical predictors of the sex of child were evaluated by the generalized linear model (GLZ). Binomial distribution and logit link function, as recommended by Wilson and Hardy (2001), were used for the construction of the model. The logistic regression subroutine was used to evaluate the relationship between the sex ratio and $\log_2$-transformed anti-*Toxoplasma* antibodies titre values. The relation between sex ratios and the expensiveness of particular clinics in Prague and in Czech Republic was tested with nonparametric Kendall test. In GLZ and logistic regression, *F*-statistics, obtained by dividing log-likelihood ratio chi-square by degrees of freedom of the effect, with number of mothers minus effect *df* minus 1 as the error *df*, were further used for computing significance corrected for pseudoreplications, resulting from the fact that some mothers gave birth repeatedly or gave birth to twins (Krackow and Tkadlec 2001). The ordered heterogeneity test (Rice and Gaines 1994) was used for testing the directional hypothesis on the relation between expensiveness of clinics and observed sex ratio. The partial Spearman test (controlled for prevalence of toxoplasmosis in particular clinics) was done as described in Sheskin (2003). The statistical program Statistica 6.0 was used for all testing.

Results

We examined the sex ratio for a sample of 1,803 infants born in three maternity clinics in Prague, whose mothers had been serologically tested for the presence of antibodies against *Toxoplasma* by either an indirect IIFT or a CFT. The proportion of males in all three sets together [0.547, N=1,803, C.I.$_{95}$=(0.524, 0.570)] was higher than the average proportion of males born in mostly toll-free state clinics in the entire Czech Republic (0.514, N=93,685) and usually toll-free clinics in the capital of Prague (0.519, N=10,057), the highest being in the two most expensive private Prague clinics (0.548, N=758 and 0.601, N=276, 5,000 Kč/pregnancy) and intermediate in the cheaper private Prague clinic (0.525, N=769, 500 Kč/pregnancy). The Kendall test showed positive correlation between the sex ratio and the fee in these five sets (tau=0.949, p=0.020).

The sex ratio was higher in 454 *Toxoplasma*-positive mothers [proportion of males, 0.608; C.I.$_{95}$=(0.563, 0.653)] than in 1,349 *Toxoplasma*-negative mothers [proportion of males, 0.527; C.I.$_{95}$=(0.500, 0.554), p=0.0027]. The effects of *Toxoplasma* positivity (binary), maternal age (continuous), primiparity (binary) and clinic (categorical) on the sex of the newborns (binary) were studied with GLZ. The final model obtained with backward stepwise selection included the variables *Toxoplasma* positivity (χ^2=10.183, p=0.001) and

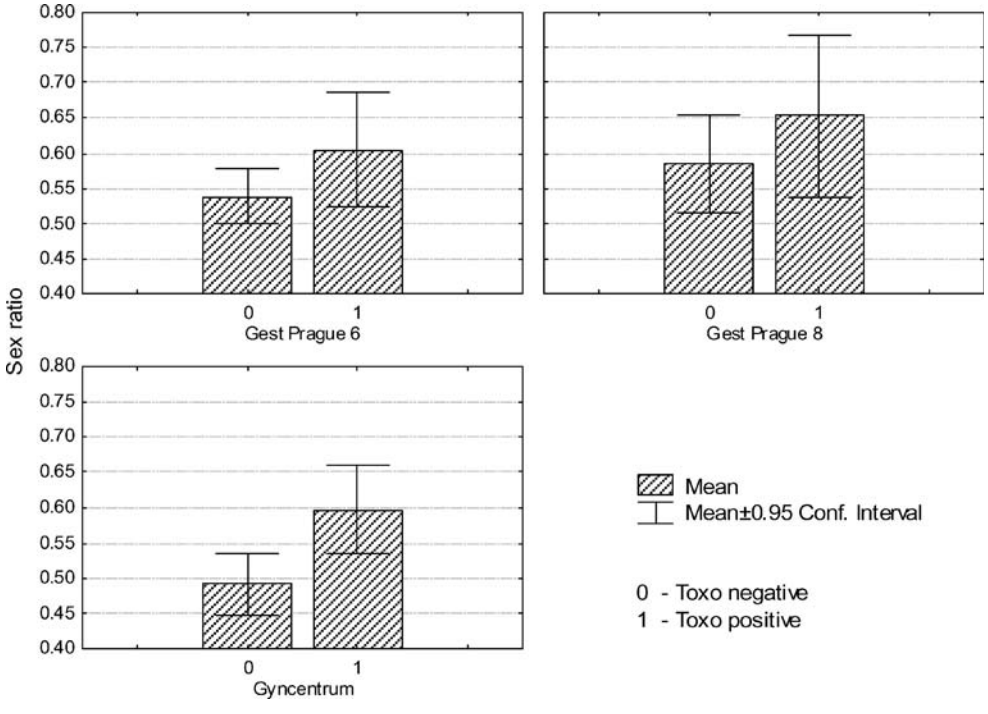

Fig. 1 Sex ratios (±0.95 C.I.) for *Toxoplasma*-negative and *Toxoplasma*-positive women in three private Prague clinics: Gest Prague 6 (619 *Toxoplasma*-negative women and 139 *Toxoplasma*-positive women), Gest Prague 8 (207 *Toxoplasma* -negative women and 69 *Toxoplasma*-positive women) and GynCentrum (523 *Toxoplasma*-negative women and 246 *Toxoplasma*-positive women)

clinic (χ^2=6.084, p=0.048). After the correction for pseudo-replications, the effect of *Toxoplasma* positivity on the sex ratio (Fig. 1) remained significant [$F_{1,\ 1,408}$=10,162, p=0.001, odds ratio=1.426, C.I.$_{95}$=(1.145, 1.776)], while the effect of clinic turned nonsignificant ($F_{1,\ 1,407}$=2,971, p=0.085). This value turned significant when the information on the rank order was taken into consideration using ordered heterogeneity test (r_sP_c=0.792, p=0.032, without filtering the effect of toxoplasmosis; $r_sP_{c\ partial}$=0.915, p=0.015, with filtering the effect of toxoplasmosis with partial Spearman correlation).

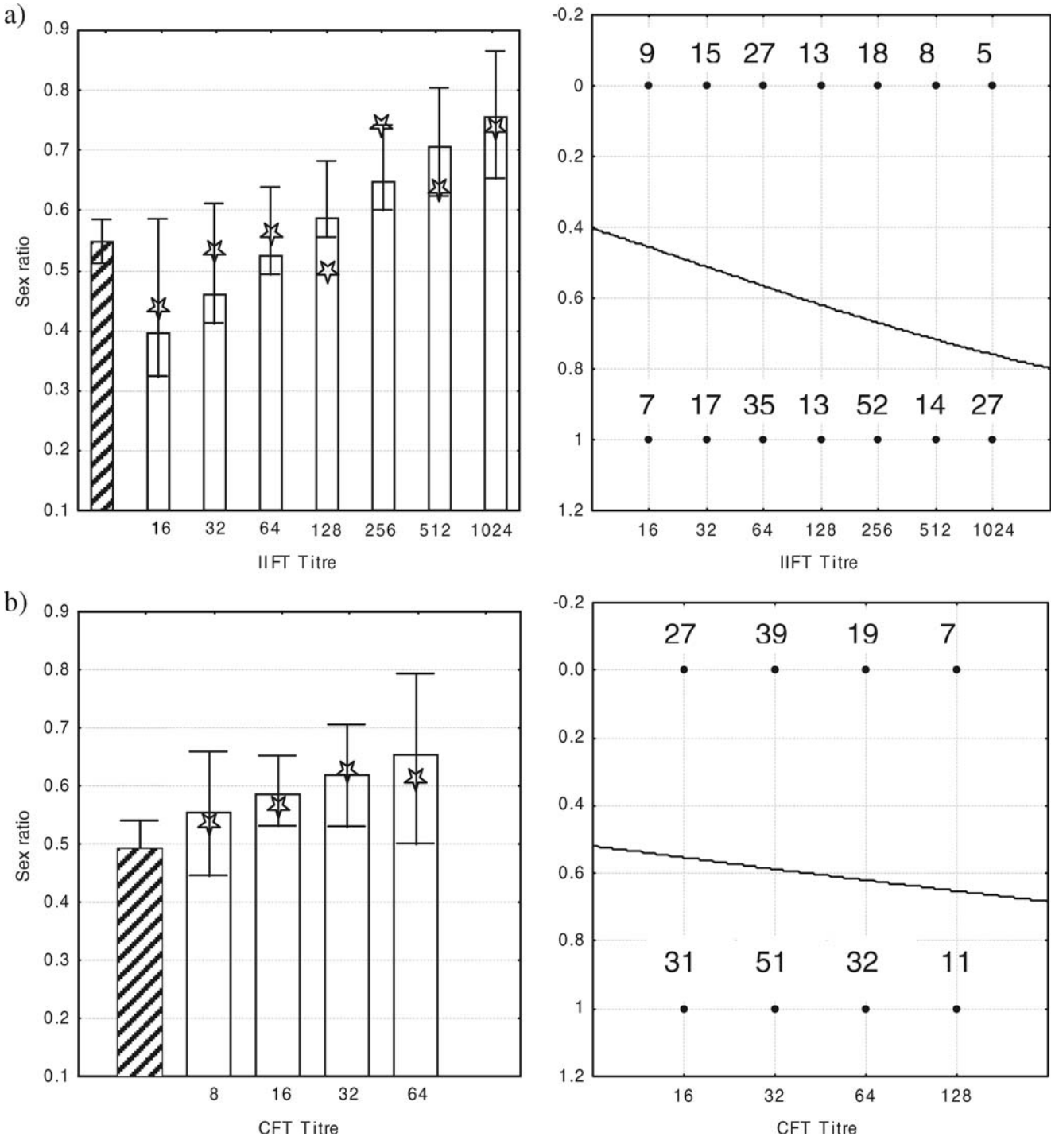

Fig. 2 Relationship between the secondary sex ratio and the concentration of anti-*Toxoplasma* antibodies in *Toxoplasma*-positive women tested by **a** IIFT (247), **b** CFT (225). The first (*shaded*) column shows the secondary sex ratio in *Toxoplasma*-negative women tested by IIFT (**a**) and tested by CFT (**b**). *Error bars* indicate 0.95 C.I. for the forecasted value, and the *asterisks* indicate the real values of sex ratio for a particular titre. The numbers in the *right panels* show number of male (1) and female (0) offspring in particulcar category

The probability of the birth of a male child increased with the increasing IIFT titre of anti-*Toxoplasma* antibodies (N=247, $F_{1,\ 213}$=7.389, p=0.007; Fig. 2), up to a value of 0.72 C.I.$_{95}$=(0.636, 0.805) for 111 mothers with the highest titres (>128). For the CFT-tested women, the relation was not significant (N=225, $F_{1,\ 176}$=0.805, p=0.371; Fig. 2), which was probably due to the low discriminating power of this serological test.

Discussion

The increase of chance of giving birth to a son up to 0.72 [C.I.$_{95\ \text{forecast}}$=(0.636, 0.805)] for women with the highest antibody titres suggests that the effect of latent toxoplasmosis on the human secondary sex ratio is probably unusually strong in comparison with the reported effects of other factors in humans (James 1996; Renkonen et al. 1962; Chacon-Pugnau and Jaffe 1996). The male-biased sex ratio in infected hosts (under normal conditions of rodents) could be either just a by-product of other activities of *Toxoplasma* in a host's organism or a result of the manipulation activity of the parasite, aimed to increase production of congenitally infected male hosts. Males cannot further transmit *Toxoplasma* to the progeny but may be eaten by a cat or other felines who are the definitive host of *Toxoplasma*. As males of most rodent species are more exploratory (Frynta 1994), possess larger home ranges (e.g. Brown 1969), exhibit more aggression (Čiháková and Frynta 1996; Frynta et al. 2005) and consequently (Krackow 2005) are more migratory (Pocock et al. 2005) than conspecific females, we can speculate that overproduction of males by *Toxoplasma*-infected females may considerably facilitate long-range transmission of the infection in natural situation.

At present, we can also just speculate about the proximal mechanism possibly underlying the sex ratio shift. Toxoplasmosis is accompanied by the production of immunosuppressive lymphokines interleukin-10 and transforming growth factor-beta (Filisetti and Candolfi 2004). Also, nonspecific stress axis activation is known to result in systemic immunosuppression (Elenkov and Chrousos 2002). The implantation success rate is higher for male zygotes (Kirby et al. 1967; Kirby 1970), and consequently, the sex ratio at the early embryonal stage (0.621 in weeks 5–7 of gestation) is heavily male-biased (Kellokumpu-Lehtinen and Pelliniemi 1984; Evdokimova et al. 2000; Milki et al. 2003; Vatten and Skjaerven 2004). The maternal immunological reaction against male-specific H-Y antigens is an important cause of selective mortality of the Y-chromosome-bearing embryos and, consequently, of the secondary sex ratio adjustment (Christiansen et al. 2004). The toxoplasmosis-associated immunosuppression or immunomodulation can be responsible for the enhanced survival of male embryos in the infected women.

The increased sex ratio in clients of expensive private clinics [0.547, C.I.$_{95}$=(0.524, 0.570), the phenomenon occurring in both infected and noninfected women and therefore independent on the effect of toxoplasmosis] reminds us of the Trivers–Willard hypothesis (Trivers and Willard 1973) and observations presented by Grant (1998) concerning a male-biased sex ratio in high-status females. The sex ratio in small- and moderate-sized samples is known to fluctuate from place to place and from time to time. For example, the data published by the Czech Statistical Office show that the sex ratio in 80 districts of the Czech Republic fluctuates from 0.48 to 0.54. However, the value 0.547 in our relatively large sample and the positive correlation between the fee and sex ratio are, at the very least, suspicious.

The prevalence of latent toxoplasmosis in women of reproductive age varies among countries (e.g. 23% in Italy, 39% in Germany, 42% in Spain and in Austria, 50% in Belgium and 54% in France; Tenter et al. 2000). An absence of a strong correlation between the sex ratio and the prevalence of toxoplasmosis in particular countries (results not shown) might be explained by an existence of negative feedback between the risk of *Toxoplasma* infection and the level of anti-*Toxoplasma* antibodies in women of reproductive age. In high-prevalence countries, most women of reproductive age have already been infected for a long time and therefore have only low titres of anti-*Toxoplasma* antibodies. Our results suggest that low-titres women have similar sex ratios to *Toxoplasma*-negative women.

It is not possible to distinguish between the cause and effect on the basis of observational study. The criterion of temporality (infection predates the pregnancy) and the criterion of a biological gradient (positive correlation between the effect size and concentration of anti-*Toxoplasma* antibodies) suggest rather that toxoplasmosis is the cause of the sex ratio shift. However, an independent confirmation of this tentative conclusion by a manipulative experiment (by experimental infection of animals) is necessary.

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