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The effects of toxoplasmosis on sex ratio at birth

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ABSTRACT

Toxoplasmosis affects about one third of human population worldwide. It has a wide range of effects on the health, immunity, behaviour, and both prenatal and postnatal outcomes of infected hosts, including humans. Among these effects, stage of infection-specific shifts in secondary sex ratio were described about ten years ago both in humans and in artificially infected mice. In both women and female mice, in the early stage of infection the probability of giving birth of sons significantly increases, up to 260 sons to every 100 daughters. In the late stages of infection, the probability of giving birth to sons markedly decreases to as low as 78 to every 100 daughters.

An ecological correlation study shows that the effect of latent toxoplasmosis on human population biology and demography can be large. In fact, the effect of prevalence of toxoplasmosis on a nationwide sex ratio was the third strongest effect from the effects of 15 factors included in the analysis. It has been suggested that toxoplasmosis-associated concentration of steroid hormones or glucose may be the proximal cause in the sex ratio shift. A more parsimonious explanation of the upward secondary sex ratio shift is found in a lower stringency of quality control of embryos, whose side-effect is increased survival rate of the more immunogenic male embryos in immunosuppressed infected females. The most parsimonious explanation of the downward secondary sex ratio shift relies on the Trivers-Willard hypothesis, which predicts an adaptive shift to more daughters in females with impaired health or lower socioeconomic status.

1. The effects of latent toxoplasmosis on the host organism

Toxoplasma gondii is a protozoan parasite of felines, which uses as its intermediate host any warm-blooded animal, including humans [1]. It is estimated that worldwide, about one third of human population is infected by *T. gondii*. After about 1–2 months of acute infection, toxoplasmosis in most subjects with intact immune system spontaneously proceeds into a latent phase. Once infected, people carry infectious tissue cysts of the parasite throughout their life [2]. Intensive research of human infection started in mid-twentieth century. This soon led to the recognition of some clinically important forms of the infection, especially ocular toxoplasmosis, which can lead to significant visual impairments including blindness, and congenital toxoplasmosis, which can result in abortion or serious developmental defects [3]. Nevertheless, the most common form of the disease, lifelong latent toxoplasmosis, has been considered asymptomatic until the beginning of our millennium.

In the 1990s, there appeared the first studies which showed that subjects with latent toxoplasmosis have a different personality than their non-infected peers [4,5]. In the first decade of the twenty-first century, it has been demonstrated that infected subjects also differ in behaviour, have longer reaction times, and correspondingly are at a higher risk of being involved in traffic accidents [6]. They score lower in many performance tests and their condition is associated with increased risk of certain psychiatric and neurological disorders. Over one hundred studies confirmed the influence of latent toxoplasmosis on the risk of schizophrenia [7–10]. More recent studies had also revealed strong positive associations between latent toxoplasmosis and

obsessive-compulsive disorder, autism, anxiety disorders, learning disabilities, and several other neuropsychiatric disorders [11–13].

It has been proposed that the proximal cause of behavioural changes is to be found in the complex changes in the concentration of immunomodulators, hormones, and neurotransmitters, especially dopamine [14]. At first, most of these changes were thought to be due to parasitic manipulation aimed at changing host behaviour in ways that would increase the chance of transmission of the parasite from an infected host to the definitive host by predation. Nevertheless, analyses of psychological changes associated with latent toxoplasmosis revealed that most of these changes are in fact side-effects of gender-specific behavioural coping with mild but chronic stress caused by lifelong chronic infection [15]. The stress coping hypothesis found further support in recent findings which showed that *Toxoplasma*-infected subjects scored worse on 28 out of 29 monitored health-related variables and suffered from a higher incidence of 77 of 134 studied diseases [16]. An ecological correlation study, meanwhile, showed that differences in toxoplasmosis prevalence could explain approximately 23% of between-countries differences in total disease burden in Europe [17].

2. The effect of latent toxoplasmosis on the reproductive functioning of hosts

In the past twenty years, various studies reported a wide range of effects of latent toxoplasmosis on the reproductive functioning of host humans. Women with latent toxoplasmosis have slower foetal development at estimated sixteenth week of pregnancy and longer pregnancies [18,19], elevated level of glucose in oral glucose tolerance tests

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and correspondingly higher incidence of gestational diabetes mellitus [20], are at a higher risk of autoimmune thyroid diseases [21], and exhibit increased levels of thyroid peroxidase antibodies and decreased levels of free thyroxine in the serum [22]. Women with latent toxoplasmosis also gain more weight in pregnancy. This increased weight gain in the early stages of pregnancy in Rh-negative subjects is probably the strongest effect of latent toxoplasmosis described in scientific literature: by the sixteenth week of pregnancy, *Toxoplasma*-infected Rh-negative women tend to gain about twice more weight than *Toxoplasma*-free or Rh-positive women (4.12 kg compared to 2.50 kg), although in size (weight and length) their foetuses and newborn infants are approximately the same [23].

Several studies show that women with latent toxoplasmosis experience fertility problems more frequently than non-infected controls [24,25]. *Toxoplasma*-infected women reported that it took them longer to conceive and they more frequently turned to assisted reproductive technologies [26]. A relatively recent study by Salman [27] reported a significant relationship between toxoplasmosis and anti-Mullerian hormone level as a predictor of ovarian reserve in females, while another team demonstrated a significantly higher seroprevalence of IgG antibodies against *T. gondii* in women with recurrent miscarriage (42.1%) than in controls (25.1%) [28].

Possibly the most noticeable effect of latent toxoplasmosis on human reproductive function, however, is its impact on the secondary sex ratio. Studies which investigated three different populations of women showed that women with latent toxoplasmosis had a much higher secondary sex ratio (SSR), i.e. more sons, than non-infected women [29]. Detailed analyses, however, revealed that in *Toxoplasma*-infected women with lower concentrations of anamnestic anti-*Toxoplasma* antibodies (and presumably correspondingly old infections), the probability of giving birth to a son was lower than 0.5. In particular, it was 0.4 for women with the lowest but still positive indirect immunofluorescence titres (IIFT), i.e. those whose IIFT was 16, and 0.46 for women with the second lowest positive titres (IIFT titre of 32). With increased concentration of anti-*Toxoplasma* antibodies, the SSR increased up to 0.72 (C.I.₉₅ = [0.636, 0.805] for 111 mothers with IIFT titres over 128). This means that for every 260 boys, 100 girls are born to women with the highest concentration of anti-*Toxoplasma* antibodies and therefore the most recent *Toxoplasma* infection.

It has been speculated that toxoplasmosis is not the cause of the observed SSR shift. In theory, for instance, women with a lower capacity of the immune system or specifically immunomodulated immune system could be both an increased risk of *Toxoplasma* infection and have a higher chance of having sons. This hypothesis, however, was rejected because two independent experiments performed on laboratory animals showed that two to three months after peroral infection with *Toxoplasma* bradyzoites, laboratory mice had a significantly higher SSR (0.66 and 0.55 in infected mice versus 0.32 and 0.46 in controls in the first and second experiment, respectively) [30]. In contrast, mice that gave birth 121 and 156 days after being infected had a lower SSR (0.41 and 0.38 in infected mice versus 0.59 and 0.55 in controls in the first and second experiment, respectively).

Recently, similar results were obtained in the cross-sectional study performed in Tehran in 2014–2015 [31]. In this study, 850 cord blood samples were analysed by enzyme-linked immunosorbent assay. Results showed that *Toxoplasma*-seropositivity was significantly associated with having a male offspring (OR = 1.64). In particular, 103 out of 166 (62.1%) infants born to seropositive mothers were male, while 341 out of 684 (49.9%) infants born to seronegative mothers were male. The OR of having male offspring increased up to 2.10 in seropositive women with a high concentration of anti-*Toxoplasma* antibodies (optical density > 0.75) compared to that in *Toxoplasma*-negative group.

The effect of latent toxoplasmosis on human SSR has also been confirmed by an independent method, namely an ecological correlation study performed on 94 national populations distributed across Africa, the Americas, Asia, and Europe, for which both the SSR and the

prevalence of toxoplasmosis in women of childbearing age had been published [32]. A statistical analysis had shown that the prevalence of toxoplasmosis is probably the most important environmental factor which influences the SSR ($\beta = -0.097$, $P < 0.01$) and the third most important factor of those followed in the study immediately after a social factor of son preference ($\beta = 0.261$, $P < 0.05$) and the number of children per women ($\beta = -0.145$, $P < 0.001$).

The abovementioned study investigated the effects of number of children per women, maternal age, polygyny intensity, wealth, son preference, latitude, parasite stress, nutritional stress, contraceptive use and health status, humidity, sanitation rate, cat ownership, meat consumption, and toxoplasmosis prevalence. A negative correlation between toxoplasmosis prevalence and the SSR was detected in all 94 countries, including 64 non-European countries, although in 30 European countries, the correlation was not significant. This pattern was expected based on the results of previous human and animal studies.

One could hypothesise that in high-prevalence countries (which were mostly the non-European ones), most women tend to be infected with *T. gondii* early in life, probably by contact with oocyte-contaminated soil or by eating insufficiently cooked meat containing tissue cysts of *T. gondii*. By the time these women reach childbearing age, they have therefore chronic infection and that is associated with lower SSR. In low-prevalence countries, on the other hand, many women are infected with *T. gondii* much later, possibly even shortly before conceiving, and quite possibly by sexual transfer from infected partners [33]. The increased SSR in recently infected women can neutralise or even reverse the negative correlation between toxoplasmosis prevalence and the SSR observed in older cases of latent toxoplasmosis. Such an effect in recently infected women could be especially strong in low-fertility (European) countries, where women start bearing children later in life but most women also stop reproducing earlier, namely after the birth of their first or second child. The probable duration of *Toxoplasma* infection positively correlates with maternal age and therefore also with parity. Consequently, women in low-fertility countries tend to have (on average) younger infections than women in high-fertility countries. The results of this (so far) only published ecological study provide strong support for the existence (and global importance) of the effect of latent toxoplasmosis on the SSR in human populations. Nevertheless, its results should be confirmed by similar studies that would investigate particular regions of large countries, such as the USA, Mexico, France, or the UK, for which data on *Toxoplasma* prevalence as well as on SSR are available on national level.

3. Probable mechanisms of the upward SSR shift associated with recent *Toxoplasma* infections

Several mechanisms that might be responsible for the effects of toxoplasmosis on the SSR have been suggested. Increased levels of glucose in infected women [20] could lead not only to the reported increased risk of gestational diabetes mellitus but also to an increased SSR [34,35]. Alternatively, it has been shown that *Toxoplasma*-infected men [36], women [37] and male rats [38] have significantly higher levels of testosterone, whereby it is known that the likelihood of having a son positively correlates with testosterone concentrations in both men and women [39]. On the other hand, however, other studies have demonstrated that infected women [36] and female mice had lower testosterone levels [40] than their non-infected female peers. In fact, lower testosterone levels were even observed in infected male mice [40] and men [41]. In the light of these findings, the testosterone-based explanation of positive SSR shift associated with the early stages of latent toxoplasmosis must be approached with a caution.

Other possible explanations of the increased SSR in women with recent infections takes into account the observed immunosuppressive or immunomodulating effects of latent toxoplasmosis in men and animals [42]. It is known that leukocyte, NK-cell, and monocyte counts decrease

in *Toxoplasma*-infected male subjects and increase in *Toxoplasma*-infected female subjects, while B-cell counts are reduced in both *Toxoplasma*-infected males and females. Moreover, these changes become less pronounced with decreased concentrations of anamnestic anti-*Toxoplasma* titres of IgG antibodies, i.e. they abate as time passes since the acquiring the infection. This decrease is reminiscent of the pattern of the negative effect of latent toxoplasmosis on the SSR. The direction of causality of the observed changes in the immunity status of infected individuals has been confirmed by laboratory infection of mice [43–45]. Mice in the early stage of latent infection exhibited a temporarily increased production of interleukin 12 and decreased production of interleukin-10. Throughout the early and late stage of latent toxoplasmosis, the infected animals showed a decreased production of IL-2 and nitric oxide and decreased proliferation reaction (synthesis of DNA) in a mixed lymphocyte culture.

The immunosuppression hypothesis [29] suggests that the immunosuppressive effect of recent toxoplasmosis infection could lead to increased SSR by protecting the more immunogenic male embryos, which contain so called Y-antigens on their surface, against miscarriage. In humans, 1.64 times more male than female embryos start developing in the uterus [46]. Nevertheless, partly due to higher immunogenicity and partly due to a higher incidence of developmental defects in male embryos [47], many are aborted in a process of ‘quality control’. By inducing immunosuppression, *Toxoplasma* could lower the stringency of this quality control, which would result in better survival chances of male embryos and therefore also in increased SSR in women recently infected with toxoplasmosis. One of the most comprehensive and largest studies focused on sex ratio during pregnancy showed that between the conception to delivery, the sex ratio fluctuates rather than uniformly decreases [48]. Contrary to the frequent claim that the sex ratio at conception is male-biased, data analysis suggested that sex ratio at conception is 0.5. Authors described the trajectory of human sex ratio from conception to birth by analysing data from (i) 3- to 6-days-old embryos, (ii) induced abortions, (iii) chorionic villus sampling, (iv) amniocentesis, and (v) foetal deaths and live births. The sex ratio may decrease in the first week or so after conception (due to excess male mortality), then it increases for at least 10–15 weeks (due to excess female mortality), levels off after approximately week 20, and slowly declines from week 28 to 35 (again due to excess male mortality). Finally, the sex ratio among abnormal embryos is male-biased. The whole picture is therefore probably more complex than has been originally supposed, but it is still in line with the proposed immunosuppression hypothesis.

The existence of an effect of early-stage latent toxoplasmosis on the stringency of the embryo quality control is supported by the results of older studies. In fact, it has been shown nearly 70 years ago that among mothers of children with the Down syndrome, seroprevalence of toxoplasmosis was about 84% (63% strong reactions in intradermal delayed hypersensitivity test [IDHT], i.e. recent infections, 21% of weak reactions in the IDHT, i.e. older infections) [49]. This was significantly more than in the general population of the same age range, where toxoplasmosis prevalence was 32%. Of children with the Down syndrome, 42% were IDHT positive, whereas in the general population of the same age range, it was only 13%. Except for one case, toxoplasmosis-free mothers did not have toxoplasmosis-positive children, while infected children in all cases had infected mothers. Noninfected children's mothers may but need not have had toxoplasmosis. Fathers of children with the Down syndrome had approximately the same prevalence of toxoplasmosis as the general population. The authors of this study did not at the time suggest any explanation for this paradoxical phenomenon, but it seems quite probable that lower stringency of quality control may have been responsible for the higher survival of embryos with a genetic defect, in this case Trisomy 21.

The survival of a higher proportion of embryos with mild developmental defects could also be responsible for the observed slower development of embryos in infected women [18] and for slower early

postnatal development of infants born to them [50]. Toxoplasmosis could weaken or switch off the mechanism of spontaneous abortions, which is under normal conditions responsible for the elimination of embryos with genetic or developmental disorders often associated with a (statistically) slower foetal growth rate and slower early postnatal development.

4. Probable mechanisms of the downward shift in SSR associated with latent toxoplasmosis

At least two possible mechanisms might explain the observed decrease of SSR in women in the late stages of toxoplasmosis. As mentioned above, infected women have a lower and infected men a higher concentration of testosterone than the noninfected controls [36,51]. There are even some indirect indications to the effect an early increase in testosterone levels in infected men is followed by testosterone concentration decrease in the later stages of infection. For example, one study found that infected men exhibit a narrower repertoire of sexual behaviours [52], while another showed that in infected men, there is an increased probability of preference for sexual submission [53]. Moreover, studies which showed increased testosterone levels in infected men were based on a sample of undergraduates, i.e. on a uniformly young male population. It is well possible that an opposite shift would be observed if the study were to be conducted in an older population. In mice, both infected males and females have decreased testosterone levels [40], while in rats, increased testosterone levels were observed only in fertile males but not in females or castrated males [38].

It is known that in both males and females, a higher concentration of testosterone or other steroid hormones positively correlates with the probability of conceiving male offspring [39,54]. James [55] suggested that *T. gondii* may preferentially infect individuals with higher oestrogen levels (such individuals are more likely to acquire any infection due to the interference of high level of steroids with activity of the immune system) and that could be responsible for the observed increase in SSR in the earlier phase of the infection. In later stages of the infection, steroid hormone levels of infected hosts decreases due to infection-related pathological processes, which may be responsible for the decreased SSR in the later phases of the infection. It must be noted, however, that only indirect evidence for such gradual decreases of testosterone exists in men and no evidence for a gradual decrease in the levels of testosterone or other steroid hormones is available for women.

Another, and more clinically relevant, explanation relies on the existence of the so-called Trivers–Willard effect [56] in conjunction with the observed impaired health of subjects with latent toxoplasmosis noted above. *Toxoplasma*-infected subjects suffer from many disorders and are therefore continuously exposed to more or less severe chronic stress. It is known that women – as well as females of other species – who are in poor health or live in unfavourable socioeconomic circumstances give birth to more daughters [57], while women in good health and better socioeconomic conditions have more sons [58,59]. This is because in most species, the variance in male fecundity is much higher than the variance in females. Consequently, females are likely to have a similar number of offspring regardless of their health or socioeconomic status. In males, however, differences in the fecundity can be enormous. It is therefore advantageous for females to use a conditional reproduction strategy: to bear more sons when they have enough resources and are in good health and daughters when they are in poorer health or have less resources.

5. Toxoplasmosis-associated sex ratio shifts: evolutionary adaptations or merely side effects?

The ultimate cause of the downward shift in SSR associated with latent toxoplasmosis has also been subject of theorising. In many species, toxoplasmosis is easily transmitted vertically from mother to offspring. It has been suggested that under certain conditions, it is more

useful for the parasite to increase the number of males (as in the early stages of toxoplasmosis infection), because they are more mobile [60] and can therefore spread the infection to more distant populations [29]. Admittedly, however, similar post hoc explanations could be suggested for almost any biological phenomena and therefore their explanatory power seems rather low. One could for instance claim that decreased SSR can be viewed as a biological adaptation of *T. gondii* to species with a vertical mother-to-offspring transmission of the infection. In some species, such vertical transfer occurs regularly [61]. In these species, females should be in theory more valuable for the parasite than the males because they can transmit the infection not only horizontally to a definitive host by predation but also vertically to their offspring.

Given the current state of our knowledge, however, the most parsimonious explanation of the effects of latent toxoplasmosis on SSR should be viewed as most likely to be true. In other words, this is a situation where Occam's razor should be applied. In this case, it seems most likely the increased SSR in females recently infected with toxoplasmosis is a side-effect of decreased stringency of quality control of foetuses. That itself could be due to parasite-induced immunosuppression, which primarily protects the parasite against the host's immune system. Decreased SSR in females with older latent toxoplasmosis, i.e. those who have been infected for a long time, could then be the result of their impaired health and adoption of alternative reproduction strategy described by the Trivers–Willard hypothesis.

6. Conclusions

Latent toxoplasmosis affects about one third of human population throughout the world and its effects on the behaviour and physiology of infected hosts are significant and varied. The impact of toxoplasmosis on secondary sex ratio in humans is relatively strong: it is possibly the third most influential factor affecting this aspect of reproduction in our species. In the past, several mechanisms of the observed sex ratio shift have been proposed, some of which with potentially large clinical implications. It is therefore critically important to repeat the original human and animal case-control studies on independent populations and to undertake further ecological correlation studies on different sets of countries or districts for which both sex ratio data and toxoplasmosis prevalence data are available. Similarly, it would be most desirable to test the suggested hypotheses of the mechanism of the sex ratio shift in new experimental studies.

Author's roles

Drafting the article or revising the manuscript critically for important intellectual content (ŠK, JF), writing of the manuscript text (ŠK, JF). Both authors approved the final version of the article and agree to be accountable for all aspects of the work.

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Declaration of competing interest

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