Impaired health status and increased incidence of diseases in *Toxoplasma*-seropositive subjects – an explorative cross-sectional study

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SUMMARY

The global seroprevalence of latent toxoplasmosis is estimated to be higher than 30%. The presence of slowly dividing parasites in tissue cysts located mainly in immunoprivileged organs was long considered asymptomatic. Recently, many studies have shown that latent *Toxoplasma* infections could have serious impacts on human health. Here we ran a cross-sectional study in a population of 1486 volunteers. The results showed that 333 infected subjects scored worse than 1153 controls in 28 of 29 health-related variables. Similarly, they reported higher rates of 77 of a list of 134 disorders reported by at least 10 participants of the study. Toxoplasmosis was associated most strongly with musculoskeletal ($\tau = 0{\cdot}107$, $P < 0{\cdot}0005$), followed by neurological ($\tau = 0{\cdot}088$, $P < 0{\cdot}0005$), immune ($\tau = 0{\cdot}085$, $p < 0{\cdot}0005$), metabolic ($\tau = 0{\cdot}079$, $P < 0{\cdot}0005$), respiratory ($\tau = 0{\cdot}068$, $P = 0{\cdot}0001$), allergic ($\tau = 0{\cdot}053$, $P = 0{\cdot}004$), digestive system ($\tau = 0{\cdot}052$, $P = 0{\cdot}004$) and mental health disorders ($\tau = 0{\cdot}050$, $P = 0{\cdot}008$). Results of the present cohort study, along with the previous data from many case-control studies or ecological studies suggest that latent toxoplasmosis represents a large and so far underrated public health problem.

Key words: Parasite, public health, disease burden, neglected disease, neglected zoonosis, toxoplasmosis, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii infects about one third of inhabitants on Earth (Tenter *et al*. 2000; Pappas *et al*. 2009). In many countries, including several highly developed ones like France and Germany, more than 50% of the population acquire *T. gondii* infection during their lifetime (Pappas *et al*. 2009). Acute toxoplasmosis promoted by rapidly dividing tachyzoites has a mostly subclinical course with minor symptoms in immunocompetent subjects followed by latent stage (Montoya & Liesenfeld, 2004). It is, however, the most common food-borne parasitic infection requiring hospital treatment in France (Vaillant *et al*. 2005), the third most common cause of hospitalization due to food-borne infections (Mead *et al*. 1999) and one of the leading causes of death attributed to food-borne illness (Scallan *et al*. 2011). About one million new infections are estimated to occur each year in the USA, which result in 20 000 cases of retinal pathology (Jones & Holland, 2010). Latent toxoplasmosis can be defined as the presence of slowly dividing bradyzoites of *Toxoplasma* in tissue cysts localized mostly in the immunoprivileged organs, namely the brain, eye and testes. It is generally believed that latent toxoplasmosis, accompanied by fluctuating anamnestic levels of anti-*Toxoplasma* IgG antibodies, is a lifelong condition (Tenter *et al*. 2000). The presence of anamnestic antibodies protects infected subjects against new infections. In immunosuppressed subjects, e.g. AIDS patients or artificially immunosuppressed transplant recipients, toxoplasmosis can reenter the acute phase and without proper treatment, can lead to life-threatening cerebral toxoplasmosis (Porter & Sande, 1992; Akanmu *et al*. 2010; Addebbous *et al*. 2012). For a long time, latent toxoplasmosis was considered clinically asymptomatic. About 20 years ago, behavioural manifestations of toxoplasmosis such as specific changes in personality traits or prolonged reaction times were described (Flegr *et al*. 1996; Havlíček *et al*. 2001). Some of the observed changes are believed to be the result of the manipulative activity of *Toxoplasma*, the evolutionary adaptation of this parasite that increases the chances of parasites transmission from an intermediate host (any warm-blooded animal) to the definitive host (any feline species) by predation (Flegr & Hrdý, 1994; Webster, 1994). Some are probably side-effects of other activities of the parasite, such as the upregulation of the production of dopamine (Flegr *et al*. 2003; Gaskell *et al*. 2009; Prandovszky *et al*. 2011) and testosterone (Flegr *et al*. 2008; Kaňková *et al*. 2011; Lim *et al*. 2013), or of a mild chronical stress accompanying latent infection (Lindová *et al*. 2010, 2006).

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In the past 10 years, many associations between latent toxoplasmosis and certain health disorders have been observed and published. Infected subjects have a highly increased probability of being diagnosed with schizophrenia (Torrey *et al*. 2007, 2012), mood disorders (Pearce *et al*. 2012; Radford *et al*. 2012), epilepsy (Stommel *et al*. 2001; Palmer, 2007), autism (Prandota, 2010; Blomstrom *et al*. 2012), migraine and other headaches (Koseoglu *et al*. 2009; Prandota, 2009), melanoma (Nagineni *et al*. 2002), carcinoma of female genitals and breast cancer (Sanchis-Belenguer *et al*. 1984; Vos, 1987), heart diseases (Paspalaki *et al*. 2001; Yazar *et al*. 2006), inflammatory bowel disease (Prandota, 2012), celiac disease (Nejad *et al*. 2011; Prandota, 2012) impaired liver functions (Vethanyagam & Bryceson, 1976; Ustun *et al*. 2004), hematological changes (Flegr & Stříž, 2011), thyroid diseases (Singh *et al*. 1994; Kankova *et al*. 2014), rheumatoid arthritis (Tomairek *et al*. 1982; Torrey & Yolken, 2001; Shapira *et al*. 2012), glomerulonephritis (van Velthuysen & Florquin, 2000; Kapoor, 2012; Toporovski *et al*. 2012), diabetes (Kaňková *et al*. 2015*b*), and changes in lipid contents, including atherosclerosis (Coppens, 2006; Flegr *et al*. 2012). Infected subjects also have a higher probability of suicides (Yagmur *et al*. 2010; Ling *et al*. 2011; Pedersen *et al*. 2012) and traffic or working place accidents (Alvarado-Esquivel *et al*. 2012; Flegr *et al*. 2002, 2009). An exhaustive survey of such effects of toxoplasmosis is provided in a previous article by our group (Flegr *et al*. 2014). In that study, the existence of most of the already known associations, as well as many new ones, was observed. That ecological study was performed on a set of 88 countries for which the necessary data were available. It showed that the prevalence of toxoplasmosis is correlated with the data on specific disease burden collected and published by the WHO. The study revealed that for example, morbidity of 23 of 128 analyzed diseases and disease categories on the WHO list showed correlations with the prevalence of toxoplasmosis and another 12 diseases had positive trends ($P < 0{\cdot}1$). When the confounding variables like gross domestic product (GDP) per capita, geolatitude and humidity were controlled, the prevalence of toxoplasmosis explained 23% of the variability in disease burden in Europe (Flegr *et al*. 2014).

The interpretations of ecological studies are sometimes complicated, especially if aggregated data are used for the estimation of the strength and direction of the influence of particular factors within a population (Guthrie & Sheppard, 2001; Wakefield & Salway, 2001). Results obtained in retrospective case-control studies are more reliable in this respect and can detect even weak associations between a particular health disorder and environmental factors like toxoplasmosis. However, the results performed on clinical patients could be biased by the sieve effect – for example by the unwillingness of psychiatric patients with certain forms of disorders to enter voluntarily into scientific study. Here the results are reported of an explorative cross-sectional study on a population of 1486 volunteers recruited from an internet community called Guinea Pigs and consisting of Czechs and Slovaks willing to participate in mostly evolutionary psychology experiments.

METHODS

Subjects and recruitment

The recruitment of subjects was undertaken by using the Facebook-based snowball method (Kankova *et al*. 2015*a*). To address potential volunteers, an invitation to participate in 'an experimental searching for associations of a subject' blood group and other biological factors with his/her personality, performance, morphology, and health' was posted on the Facebook wall page 'Guinea Pigs' (in Czech 'Pokusni kralici') for Czech and Slovak nationals willing to take part in evolutionary psychology experiments (http://www.facebook.com/pokusnikralici) (Flegr & Hodny, 2016). The first page of the electronic questionnaire provided information about the goal of the study. The following note was also included: 'The questionnaire is anonymous and obtained data will be used exclusively for scientific purposes. Your cooperation with the project is voluntary and you can terminate it at any time by closing this website.' The population of Guinea Pigs is 'enriched' with subjects that we serologically tested for toxoplasmosis in the past 15 years of our systematic study of behavioural effects of latent toxoplasmosis (Flegr, 2013*b*). However, most of our recent internet studies have no relation to toxoplasmosis. Moreover, toxoplasmosis was not specifically mentioned neither during the recruitment of participants nor in the informed consent to keep the study blind, and therefore to avoid a possible bias (approved by Institutional Review Board (IRB)). The first and also the final page of the questionnaire contained the Facebook share button and the following request for the participants: 'We need the highest possible number of responders. Therefore, please, share the link to this questionnaire with your friends, for example on Facebook'. The share button was pressed by 541 participants, which resulted in obtaining data from 6463 Czech and Slovak responders between 28/4/2014 and 16/11/2015. The study, including the method of obtaining the informed consent (by pressing the Next button on the first page) was approved by the IRB of the Faculty of Science (Eticka komise pro praci s lidmi a lidskym materialem Prirodovedecke Fakulty Univerzity Karlovy) No. 2014/21.

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Questionnaire

The anamnestic questionnaire was prepared by two medical doctors, *a clinician* (*internist/hematologist*) *and a researcher* (*molecular geneticist*) and was distributed as a Czech/English Qualtrics survey (http://1url.cz/q05K). It contained two categories of questions. The first of them monitored presence and intensity of general and specific health problems of responders. The responders were asked to subjectively rate of their allergic, cancer, digestive, fertility, genitourinary, heart, haematological, immunity, mental health, metabolic including endocrine, musculoskeletal, neurological, respiratory organs, sense organs and sexual life problems using 6-points Likert scales. The second group of questions tried to collect objective information reflecting the health status of responders. We asked the responders, for example, how many drugs prescribed by doctors they currently take per day, how many of 'different herbs, food supplements, multivitamins, superfoods etc.' they currently take per day, how many times they used antibiotics during the past 365 days. We also provided the responders lists of about 250 disorders (separated to 15 categories) and asked them to tick which of them they were diagnosed with. The questionnaire contained, among others, also the following questions: 'Are you infected with *Toxoplasma* (a parasite living in cats which is dangerous for pregnant women)?' with three options: (a) I do not know/I am not sure, (b) no (I was tested but I was negative) (c) yes (I was tested and I was positive – I have antibodies against this parasite). Implicitly, the answer a) (I do not know/I am not sure) was checked. The responders of our questionnaires had three options: they could complete any questionnaire absolutely anonymously, they could sign the finished questionnaire by a code obtained after anonymous registration, or they could sign the finished questionnaire by a code obtained after non-anonymous registration (see http://pokusnikralici.cz). Some questionnaires are 'signed' by less than 1% of non anonymously registered subjects (e.g. the questionnaire about sexual behaviour), some by 15% of subjects. The present questionnaire (containing sensitive information about mental health) was 'signed' by 3% of the subjects. When we checked the information about the toxoplasmosis status provided in the questionnaire by participants of our past experiments with corresponding information in our records, we found a perfect (100%) agreement.

The questionnaire contained also some questions unrelated to the topic of the present study (e.g. a short personality test) and simple tests of reaction times, operational, short-term and long-term memory, psychomotor performance and intelligence. In the present paper, however, only the health status and diseases and disorder-incidence-related questions were analyzed.

Immunological tests for T. gondii *infection*

Most of the women and nearly all of men who know their *T. gondii*-infection status were tested for *T. gondii* infection during systematic research of behavioural effects of latent *T. gondii* infection, which has been running at the Faculty of Science for 20 years. All testing was performed at the National Reference Laboratory for Toxoplasmosis, National Institute of Public Health, Prague. The complement-fixation test (CFT), which determines the overall levels of IgM and IgG antibodies of particular specificity and Enzyme-Linked Immunosorbent Assays (ELISA) (IgG ELISA: SEVAC, Prague) were used to detect *T. gondii* infection status of the subjects. ELISA assay cut-point values were established using positive and negative standards according to the manufacturer's instructions. In CFT, the titre of antibodies against *T. gondii* in sera was measured in dilutions between 1:4 and 1:1024. The subjects with CFT titres between 1:8 and 1:128 were considered *T. gondii* infected. Only subjects with clearly positive or negative results of CFT and IgG ELISA tests were diagnosed as *T. gondii*-infected or *T. gondii*-free; subjects with different results of these tests or ambiguous results of tests were retested or excluded from the study.

Data analysis

SPSS v. 21 was used for the statistical analysis. Differences in age were tested by *t*-test and differences in the prevalence of toxoplasmosis between men and women by logistic regression with the age as a confounding variable. Ordinal and binary data were analyzed by partial Kendall's correlation test, which is used to measure the strength and significance of the association between binary, ordinal, or continuous data regardless of their distributions and allows the control for one confounding variable, here the age (Siegel & Castellan, 1988; Kaňková *et al*. 2011). The Excel spreadsheet used to compute the partial Kendall tau and the significance for variables A (diseases) and B (*Toxoplasma* infection), once C (age) was controlled -based on Kendall Taus AB, AC and BC- is available at: http://web.natur.cuni.cz/flegr/programy.php. When the number of subjects reporting a disorder was less than 10, the Fisher exact test was used for studying the association between toxoplasmosis and the disorder. Correction for multiple tests was performed with Benjamini-Hochberg procedure. (Benjamini & Hochberg, 1995). In the contrast to simple Bonferroni's correction, this procedure takes into account also the distribution of p values of performed multiple tests. Therefore, when the studied factor has multiple effects, the number of significant results after the correction could be higher than before the correction.

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The prevalence of certain diseases is gender specific and also many effects of latent toxoplasmosis have been shown to differ between men and women (Lindová *et al*. 2006, 2010). Therefore, all analyses were performed separately for men and women. All raw data are available as the Supporting Information S1, available at https://figshare.com/s/64f6b0230f733e8e3aa2.

RESULTS

Descriptive statistics

Of 6463 Czech and Slovak respondents, 1486 (365 *Toxoplasma*-free males, age 34·8, S.D. 12·7, 69 *Toxoplasma*-infected males, age 34·0, S.D. 10·5, 788 *Toxoplasma*-free females, age 32,4, S.D. 11·0, and 264 *Toxoplasma*-infected females, age 36,5, S.D. 12·3) provided information about their *Toxoplasma* status. The difference in the age between *Toxoplasma*-infected and *Toxoplasma*-free subjects was significant for women ($t_{1048} = -5{\cdot}10$, $P < 0{\cdot}0001$) but not for men ($t_{430} = 0{\cdot}520$, $P = 0{\cdot}603$). The logistic regression with age and sex as independent variables showed that the prevalence of toxoplasmosis was significantly higher in women (25·1%) than in men (15·9%), odds ratio (OR) = 1·85, $P < 0{\cdot}0001$.

Intensity of health problems

Twenty-nine dependent variables (mostly ratings of particular health problems on a 1-to-6 scale, where 1 was 'no problems at all' and 6 was 'frequent or serious') were ordinal and most of them had a highly skewed distribution. Therefore, the non-parametric partial Kendall correlation test (which enables us to control for one confounding variable, in this case, the respondent's age) was used to search for the statistical association between *Toxoplasma* infection and the intensity of 15 categories of health problems (allergic, cancer, digestive, fertility, genitourinary, heart, hematological, immune, mental health, metabolic including endocrine, musculoskeletal, neurological, respiratory, sense organs and sexual disorders). Furthermore, the association of *Toxoplasma* seropositivity with other fourteen ordinal health-related variables was analyzed: the subjectively rated physical health, the subjectively rated mental health, the number of prescription drugs currently taken by the respondent per day, the number of alternative medicines such as herbs, food supplements, multivitamins, superfoods, etc. currently taken by the respondent per day, the maximum number of times per calendar year antibiotics were taken by the respondent, the number of times acute medical attention was needed by the respondent for a serious illness (not injury) that lasted more than 3 days in the past 5 years, the number of types of specialized medical doctors the respondent saw in the past 2 years, sexual desire in comparison with age-matched peers, sexual activity in comparison with age-matched peers, the frequency of feeling tired (not after exertion, e.g. not after sports), adherence if on a diet, the frequency and severity of feeling sleepy during the day, intensity of suffering from insomnia and the frequency of headaches. The results corrected for multiple tests with Benjamini-Hochberg procedure showed that the *Toxoplasma*-infected subjects of both genders altogether, the male subjects and the female subjects reported higher number or increased severity of health problems in 28, 17 and 19 of the 29 analyzed variables, respectively (Table 1). Theoretically, less than 5 of 87 statistical tests could provide false positive results. This suggests that nearly all positive results reflect a real difference in health between *Toxoplasma*-infected and *Toxoplasma*-free subjects, rather than statistical artefacts of multiple comparisons.

Infected subjects of both genders altogether, infected male subjects and infected female subjects also reported more frequent appointments with 9, 5 and 5 out of 10 medical specialists than the corresponding *Toxoplasma*-free controls, respectively (Table 2). Some variables were significantly correlated to toxoplasmosis exclusively in one gender. For instance, as to psychiatric disorders and sense organs problems were reported only by men while metabolic problems and insomnia problems only by women.

Incidence of particular diseases and disorders

Once the participants rated the intensity of the 29 health problems, they were asked to evaluate the presence or absence of specific disorders. They answered questions like 'What kind of respiratory problems are you suffering from or did you suffer from in the past?' by ticking the corresponding boxes on the list of disorders. Similarly, they also reported which medical specialists they saw in the past 2 years. The total numbers of disorders and medical specializations on the list were 211 and 10, respectively. The associations between these 211 binary variables and *Toxoplasma* infection were analyzed by the partial Kendall Tau correlation test with age as a covariate. Out of the 211 disorders, 134 were reported by at least 10 respondents. Of this subset of 134 diseases, 77 (57%) showed a significant association with toxoplasmosis in subjects of both genders altogether, 34 (24%) in men and 51 (38%) in women after the correction for multiple tests with Benjamini-Hochberg procedure (Table 3). All of these associations -except for one- were positive, i.e. the infected subject reported a higher incidence of a particular disorder. Again, some variables were significantly correlated to

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Table 1. Correlation between toxoplasmosis and health-related variables

	Both genders		Men		Women	
Disorders	τ	p	τ	p	τ	p
Allergic problems	0·053	**0·004***	0·046	0·180	0·045	**0·038***
Immune problems	0·085	**0·000***	0·091	**0·008***	0·066	**0·002***
Digestive problems	0·052	**0·004***	0·077	**0·025***	0·032	0·137*
Heart & vascular problems	0·039	**0·038***	0·084	**0·015***	0·028	0·209
Haematological problems	0·036	0·055*	0·022	0·531	0·014	0·527
Metabolic problems	0·079	**0·000***	0·025	0·478	0·060	**0·008***
Cancer	0·040	**0·031***	0·038	0·269	0·036	0·100*
Fertility problems	0·056	**0·003***	0·044	0·205	0·044	**0·047***
Genitourinary problems	0·056	**0·003***	0·058	0·103*	0·023	0·304
Sense organs problems	0·031	0·098*	0·140	**0·000***	−0·009	0·679
Neurological problems	0·088	**0·000***	0·137	**0·000***	0·058	**0·009***
Psychiatric problems	0·050	**0·008***	0·128	**0·000***	0·012	0·601
Sexual life problems	0·031	0·101*	0·050	0·156	0·034	0·125*
Musculoskeletal problems	0·107	**0·000***	0·161	**0·000***	0·076	**0·001***
Respiratory problems	0·068	**0·001***	0·054	0·127*	0·074	**0·001***
Subjective physical health	0·026	0·141*	−0·014	0·670	0·023	0·275
Subjective mental health	0·062	**0·001***	0·051	0·128*	0·051	**0·017***
Antibiotics/year	0·036	**0·044***	0·063	0·062*	0·015	0·483
Acute care/5 years	0·043	**0·017***	0·031	0·362	0·034	0·115*
Different med. specialist appointed/2 years	0·109	**0·000***	0·105	**0·002***	0·088	**0·000***
Number of prescription drugs/day	0·058	**0·002***	0·059	0·082*	0·044	**0·040***
Number of alternative medicines/day	0·081	**0·000***	0·075	**0·027***	0·061	**0·004***
Sexual desire: self *vs* peers	−0·019	0·334	0·038	0·295	−0·005	0·833
Sexual activity: self *vs* peers	−0·030	0·113*	−0·008	0·824	−0·014	0·531
Diet regimen (adherence)	0·038	**0·046***	0·032	0·370	0·027	0·237
Insomnia (severity)	0·062	**0·001***	0·021	0·547	0·061	**0·005***
Feeling sleepy (frequency)	0·064	**0·001***	0·077	**0·026***	0·046	**0·038***
Feeling tired (frequency)	0·097	**0·000***	0·126	**0·000***	0·068	**0·002***
Headache (frequency)	0·093	**0·000***	0·156	**0·000***	0·048	**0·030***

Effects of age on health status were controlled in present partial Kendall Tau test. Positivity of τ indicates that infected subjects have higher values of particular health related variables, i.e. a worse health status, or higher sexual desire and activity. The effect size is shown as τ. Significant results ($P < 0·05$) are printed in bold and results significant after Benjamini-Hochberg procedure correction for multiple tests (performed separately for all three populations, all subjects, men and women) are denoted with asterisks. P-values <0·0005 are coded as 0·000.

toxoplasmosis exclusively in one gender. For instance, as to increased frequency of migraines and anxieties were only observed in men while increased frequency of epilepsy and fasciculation only in women.

It is possible that a general population consists of two subpopulations, one healthy and one troubled with many disorders and diseases, including toxoplasmosis. This could result in a false correlation of toxoplasmosis with various disorders. If this is true, then we should see much stronger and more numerous associations between particular disorders only in the whole population than in the population consisting of *Toxoplasma*-free subjects only. To test this, we measured the correlation between those *Toxoplasma* seropositivity-associated disorders that occurred among at least in 150 subjects, both men and women (bronchitis/pneumonia, flatulence, urethral tract infection, low sexual appetency and scoliosis). As the incidence of certain disorders differs in men and women, we analyzed the associations separately for men and women. While there were strong positive correlations between all these disorders in the total population, except those between sexual appetency and bronchitis/pneumonia, urogenital infections, and scoliosis in men, we detected only certain correlations in subpopulations of *Toxoplasma*-negative subjects. For example, in men, we detected only the positive correlation of urogenital infections and bronchitis/pneumonia ($\tau = 0·10$, $P = 0·005$), flatulence ($\tau = 0·10$, $P = 0·007$) and low sexual appetency ($\tau = 0·13$, $P = 0·005$). Moreover, the correlation between scoliosis and bronchitis/pneumonia in men was negative ($\tau = -0·09$, $P = 0·015$).

DISCUSSION

Out of the 29 ordinary variables describing health problems, 28 showed a statistically significant association with *T. gondii* infection after the correction for multiple tests. For example, infected subjects reported to take higher numbers of drugs prescribed by doctors, as well as higher number of alternative

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Table 2. Differences in number of types of medical specialists appointed in the past 2 years between *Toxoplasma*-infected and *Toxoplasma*-free subjects

	Both genders					
Specialty	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	Odds ratio	*p*
Internal medicine	892	103	255	44	1·50	**0·014***
Otolaryngology	911	84	261	38	1·58	**0·001***
Neurology	937	58	259	40	2·50	**0·000***
Psychiatry	938	57	279	20	1·18	0·408
Gynecology	775	220	219	80	1·29	**0·003***
Surgery	934	61	286	13	0·70	0·098*
Infectology	978	17	287	12	2·41	**0·000***
Orthopedics	868	127	255	44	1·18	0·217*
Dermatology	841	154	246	53	1·18	0·077*
Other specialty	738	257	196	103	1·51	**0·000***
			Men			
Internal medicine	276	41	51	10	1·33	0·272
Otolaryngology	298	19	55	6	1·73	0·099*
Neurology	307	10	51	10	6·01	**0·000***
Psychiatry	310	7	58	3	2·33	0·071*
Gynaecology	317	0	61	0		
Surgery	293	24	57	4	0·87	0·679
Infectology	310	7	56	5	3·97	**0·000***
Orthopaedics	285	32	54	7	1·17	0·626
Dermatology	278	39	47	14	2·13	**0·001***
Other specialty	253	64	48	13	1·08	0·765
			Women			
Internal medicine	616	62	204	34	1·66	**0·010***
Otolaryngology	613	65	206	32	1·47	**0·007***
Neurology	630	48	208	30	1·89	**0·000***
Psychiatry	628	50	221	17	0·97	0·646
Gynaecology	459	219	158	80	1·06	0·408
Surgery	641	37	229	9	0·69	0·193
Infectology	668	10	231	7	2·03	**0·027***
Orthopaedics	583	95	201	37	1·13	0·372
Dermatology	563	115	199	39	0·96	0·872
Other specialty	485	193	148	90	1·53	**0·000***

Numbers of *Toxoplasma*-free subjects non appointed particular medical specialist, *Toxoplasma*-free subjects appointed particular medical specialist, *Toxoplasma*-infected subjects non appointed particular medical specialist, *Toxoplasma*-infected subjects appointed particular medical specialist, odds ratio and statistical significance, respectively, are shown in six columns of each section. The effect of age on health status was controlled by using bivariate partial Kendall's correlation (non-parametric) test. Odds ratio higher than 1·0 indicates a positive association of *Toxoplasma* infection with probability of visiting particular medical specialist. Significant results ($P < 0·05$) are printed in bold and results significant after Benjamini-Hochberg procedure correction for multiple tests (performed separately for all three populations, all subjects, men and women) are denoted with asterisks. *P*-values <0·0005 are coded as 0·000.

medicines such as herbs, food supplements, multivitamins, superfoods, etc. per day, higher frequency of seeking medical attention for a serious illness (not injury) that lasted more than 3 days in the past 5 years, a higher number of types of medical specialists appointed in the past 2 years, to feel tired and to have a headache more often. They also reported to have more severe or more frequent allergic, immune, digestive, heart and vascular, metabolic, cancer, fertility, genitourinary, neurological, psychiatric, musculoskeletal and respiratory disorders. Of the 134 specific disorders ticked by at least 10 respondents, 77 showed a statistically significant association with toxoplasmosis in the whole population of men and women. Strong associations were observed for example with bronchitis (but not with pharyngitis), acquired immunodeficiencies except AIDS, both diarrhoea and constipation, mononucleosis, allergies, amoebiasis, coeliac disease, weight loss, recurrent abortion, hypothyroidism, leukemia, cervical uterine cancer, tics, fasciculation, learning disabilities, depression (men), autism, osteoporosis, scoliosis and asthma. For example, the observation of 77 (57%) of significant associations of 134 tests was more than ten times the 6·7 (5%) expected false significant results, indicating that most of the study results were not simply due to multiple comparisons. Again this conclusion was formally confirmed with Benjamini-Hochberg procedure.

This study confirmed the results of a previous ecological study that has shown a strong association between the incidence of various diseases and

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Table 3. Differences in the incidence of particular disorders between *Toxoplasma*-infected and *Toxoplasma*-free subjects

	Both genders						Men						Women					
Disorder/disease	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis-	Toxo+ Dis+	OR	*P* value
Pharyngitis	356	695	95	216	1·16	0·130	129	202	23	37	1·03	0·888	227	493	72	179	1·14	0·279
Bronchitis, pneumonia	830	221	225	86	1·44	0·000*	268	63	43	17	1·69	0·014*	562	158	182	69	1·35	0·010*
Rhinitis, tonsillitis	435	616	136	175	0·91	0·446	149	182	30	30	0·82	0·286	286	434	106	145	0·90	0·519
Tuberculosis	1049	2	311	0	0·16	1·000	329	2	60	0	0·26	1·000	720	0	251	0		
Ectoparasites e.g. lice	882	169	249	62	1·30	0·004*	301	30	50	10	2·02	0·008*	581	139	199	52	1·09	0·201
Scabies	1028	23	304	7	1·04	0·883	322	9	59	1	0·66	0·478	706	14	245	6	1·25	0·760
Helminthiasis	993	58	291	20	1·18	0·376	318	13	58	2	0·88	0·742	675	45	233	18	1·16	0·462
Chronic diarrheal dis.	1006	45	298	13	0·98	0·990	317	14	57	3	1·22	0·688	689	31	241	10	0·93	0·816
Acute diarrheal dis.	889	162	254	57	1·23	0·033*	296	35	46	14	2·58	0·000*	593	127	208	43	0·97	0·940
Acquired immunodef.	1034	17	293	18	3·73	0·000*	329	2	58	2	5·66	0·113	705	15	235	16	3·20	0·000*
Flu, flu-like virus infection	331	720	104	207	0·91	0·673	96	235	15	45	1·22	0·344	235	485	89	162	0·88	0·689
Borreliosis	976	75	277	34	1·60	0·004*	310	21	52	8	2·28	0·004*	666	54	225	26	1·43	0·118
Other tick-borne dis.	1045	6	308	3	1·72	0·435	330	1	57	3	16·29	0·012*	715	5	251	0	0·06	0·335
STDs (except HIV/ AIDS)	1033	18	305	6	1·14	0·638	325	6	59	1	0·99	1·000	708	12	246	5	1·21	0·502
Hepatitis A, E	1039	12	308	3	0·86	0·382	324	7	59	1	0·85	1·000	715	5	249	2	1·18	1·000
Hepatitis B	1047	4	310	1	0·91	1·000	329	2	60	0	0·26	1·000	718	2	250	1	1·50	1·000
Hepatitis C	1047	4	310	1	0·91	1·000	329	2	60	0	0·26	1·000	718	2	250	1	1·50	1·000
Shingles	985	66	285	26	1·36	0·117	315	16	57	3	1·06	0·934	670	50	228	23	1·35	0·256
Herpes, oral or genital	764	287	217	94	1·15	0·190	258	73	50	10	0·71	0·161	506	214	167	84	1·19	0·197
Malaria	1050	1	310	1	3·39	0·405	330	1	60	0	0·50	1·000	720	0	250	1	31·67	0·258
Meningoencephalitis	1045	6	307	4	2·29	0·076*	329	2	60	0	0·26	1·000	716	4	247	4	2·90	0·215
Inflam. of the middle ear	745	306	220	91	1·01	0·729	245	86	43	17	1·13	0·570	500	220	177	74	0·95	0·909
Eye infections	918	133	271	40	1·02	0·727	306	25	56	4	0·89	0·719	612	108	215	36	0·95	0·873
Leishmaniasis	720	0	251	0			331	0	60	0			720	0	251	0		
Fungal skin infections	545	175	199	52	0·93	0·335	283	48	50	10	1·19	0·514	545	175	199	52	0·81	0·044*
Bacterial skin infections	677	43	233	18	1·43	0·014*	311	20	52	8	2·41	0·003*	677	43	233	18	1·22	0·162
Warts	462	258	162	89	1·04	0·350	221	110	37	23	1·25	0·251	462	258	162	89	0·98	0·677
Urogenital infection	583	137	196	55	1·49	0·000*	315	16	52	8	3·04	0·000*	583	137	196	55	1·20	0·124
Swollen lymph nodes	675	45	229	22	1·86	0·000*	317	14	51	9	4·00	0·000*	675	45	229	22	1·44	0·017*
Mononucleosis	624	96	206	45	1·63	0·000*	300	31	48	12	2·43	0·000*	624	96	206	45	1·42	0·004*
Amoebiasis	719	1	248	3	9·61	0·039*	331	0	60	0			719	1	248	3	8·17	0·055*
Tonsil stones	418	93	124	43	1·47	0·004*	231	37	36	4	0·71	0·324	418	93	124	43	1·56	0·004*
Other infectious dis.	998	53	279	32	2·16	0·000*	314	17	56	4	1·34	0·469	684	36	223	28	2·39	0·000*
Skin allergy	772	261	212	95	1·33	0·002*	276	49	46	14	1·72	0·018*	496	212	166	81	1·14	0·222
Food allergy	892	141	253	54	1·35	0·006*	284	41	52	8	1·07	0·819	608	100	201	46	1·39	0·007*
Respiratory allergy	677	356	186	121	1·24	0·006*	205	120	35	25	1·22	0·297	472	236	151	96	1·27	0·006*
Other allergies	959	74	280	27	1·25	0·194	302	23	54	6	1·47	0·239	657	51	226	21	1·20	0·480
Autoimmunity	967	52	275	26	1·76	0·001*	309	7	54	5	4·10	0·000*	658	45	221	21	1·39	0·123

Rheumatoid arthritis	1012	7	295	6	2·95	0·012*	314	2	58	1	2·83	0·403	698	5	237	5	2·94	0·048*
Haematological auto-immunity disorder	1014	5	298	3	2·07	0·393	314	2	57	2	5·50	0·118	700	3	241	1	1·03	1·00
Thyroiditis	949	70	272	29	1·45	0·025*	313	3	57	2	3·71	0·178	636	67	215	27	1·19	0·480
Multiple sclerosis	1014	5	298	3	2·07	0·393	316	0	58	1	59·85	0·157	698	5	240	2	1·20	1·000
Lupus	1018	1	301	0	0·31	1·000	316	0	59	0			702	1	242	0	0·26	1·000
Immunodeficiency	974	45	275	26	2·05	0·000*	308	8	57	2	1·40	0·575	666	37	218	24	1·98	0·000*
Bechterew's disorder	1016	3	298	3	3·41	0·136	314	2	59	0	0·25	1·000	702	1	239	3	8·28	0·054*
Myasthenia gravis	1017	2	301	0	0·16	0·136	316	0	59	0			701	2	242	0	0·14	1·000
Psoriasis	995	24	296	5	0·71	0·178	302	14	57	2	0·79	0·588	693	10	239	3	0·89	0·500
Other immune disorders	972	47	283	18	1·32	0·151	308	8	57	2	1·40	0·575	664	39	226	16	1·21	0·405
Stomach or duodenal ulcer	983	36	289	14	1·33	0·342	308	12	57	3	1·38	0·493	675	24	232	11	1·34	0·451
Chronic gastritis	993	26	290	13	1·72	0·043*	317	3	59	1	1·90	0·499	676	23	231	12	1·53	0·176
Liver disease	984	35	290	13	1·27	0·355	308	12	59	1	0·47	0·224	676	23	231	12	1·53	0·101
Diarrhoea	780	239	222	81	1·19	0·014*	262	58	41	19	2·10	0·000*	518	181	181	62	0·98	0·510
Constipation	853	166	241	62	1·32	0·005*	298	22	56	4	0·99	0·930	555	144	185	58	1·21	0·068*
Maldigestion, food intolerance	879	140	253	50	1·24	0·036*	289	31	53	7	1·24	0·484	590	109	200	43	1·17	0·140
Malabsorption	992	27	287	16	2·05	0·001*	315	5	55	5	5·72	0·000*	677	22	232	11	1·47	0·181
Bulimia, anorexia	993	26	288	15	1·99	0·001*	320	0	60	0			673	26	228	15	1·71	0·008*
Flatulence	864	155	236	67	1·58	0·000*	282	38	51	9	1·32	0·314	582	117	185	58	1·56	0·000*
Weight loss	993	26	285	18	2·42	0·000*	317	3	57	3	5·55	0·053	676	23	228	15	1·94	0·001*
Pyrosis reflux	843	176	231	72	1·49	0·000*	266	54	47	13	1·37	0·181	577	122	184	59	1·52	0·003*
Gall bladder attack	986	33	283	20	2·12	0·000*	314	6	59	1	0·96	1·000	672	27	224	19	2·11	0·002*
Celiac dis.	1011	8	295	8	3·43	0·000*	319	1	60	0	0·48	1·000	692	7	235	8	3·36	0·000*
Other digestive dis.	983	36	294	9	0·84	0·467	310	10	58	2	1·11	0·899	673	26	236	7	0·78	0·336
Rheumatic heart dis.	993	0	297	0			313	0	58	0			680	0	239	0		
Hypertensive dis.	972	21	290	7	1·13	0·770	303	10	58	0	0·05	0·039*	669	11	232	7	1·84	0·312
Ischaemic dis.	987	6	294	3	1·71	0·821	309	4	58	0	0·13	1·000	678	2	236	3	4·24	0·114
Cerebrovascular dis., stroke	993	0	297	0			313	0	58	0			680	0	239	0		
Viral myocarditis inflam.	993	0	295	2	70·67	0·053*	313	0	57	1	60·32	0·156	680	0	238	1	31·42	0·260
Bacterial endocarditis inflame	993	0	297	0			313	0	58	0			680	0	239	0		
Aneurysm	992	1	297	0	0·30	1·000	313	0	58	0			679	1	239	0	0·26	1·000
Other heart diseases	958	35	278	19	1·87	0·001*	299	14	54	4	1·61	0·239	659	21	224	15	2·10	0·001*
Cerebral bleeding	991	2	297	0	0·16	1·000	312	1	58	0	0·49	1·000	679	1	239	0	0·26	1·000
Excessive bleeding	982	11	292	5	1·54	0·261	312	1	58	0	0·49	1·000	670	10	234	5	1·45	0·398
Thrombosis	980	13	291	6	1·57	0·246	308	5	57	1	1·16	1·000	672	8	234	5	1·81	0·152
Embolism, vascular occlusion	990	3	296	1	1·19	1·000	312	1	58	0	0·49	1·000	678	2	238	1	1·49	1·000
Atrial fibrillation	991	2	296	1	1·75	0·544	312	1	58	0	0·49	1·000	679	1	238	1	2·85	0·453
Arrhythmia, mild	919	74	265	32	1·50	0·013*	293	20	48	10	3·06	0·000*	626	54	217	22	1·18	0·616
Arrhythmia, severe	991	2	296	1	1·75	0·544	312	1	58	0	0·49	1·000	679	1	238	1	2·85	0·453
Anaemia	874	97	250	40	1·44	0·009*	307	6	55	2	1·92	0·356	567	91	195	38	1·22	0·314

Table 3. (*Cont.*)

Disorder/disease	Both genders						Men						Women					
	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis-	Toxo+ Dis+	OR	*P* value
Polyglobulia	969	2	290	0	0·16	1·000	311	2	57	0	0·26	1·000	658	0	233	0		
High white blood cell count	958	13	281	9	2·37	0·004*	307	6	54	3	2·88	0·147	651	7	227	6	2·46	0·021*
Low white blood cell count	957	14	288	2	0·49	0·143	306	7	56	1	0·85	1·000	651	7	232	1	0·43	0·688
Other white blood cell dis.	964	7	288	2	0·99	1·000	310	3	56	1	1·96	0·489	654	4	232	1	0·76	0·688
High platelet count	969	2	290	0	0·16	1·000	313	0	57	0			656	2	233	0	0·13	1·000
Low platelet count	963	8	284	6	2·55	0·007*	310	3	57	0	0·18	1·000	653	5	227	6	3·44	0·001*
Other platelet dis.	970	1	287	3	9·52	0·040*	312	1	57	0	0·50	1·000	658	0	230	3	88·66	0·018*
Excessive bleeding	923	48	269	21	1·50	0·024*	308	5	56	1	1·18	1·000	615	43	213	20	1·35	0·145
Excessive blood clotting	956	15	284	6	1·36	0·285	311	2	57	0	0·26	1·000	645	13	227	6	1·32	0·307
Atypical immunoglobulins	969	2	289	1	1·76	0·544	313	0	56	1	61·39	0·154	656	2	233	0	0·13	1·000
Iron deficiency	846	125	242	48	1·34	0·015*	308	5	53	4	4·66	0·035*	538	120	189	44	1·04	0·852
Swollen lymph nodes	960	11	278	12	3·76	0·000*	310	3	55	2	3·81	0·171	650	8	223	10	3·63	0·000*
Haemoglobinopathy	971	0	288	2	70·78	0·053*	313	0	57	0			658	0	231	2	59·80	0·068*
Vitamin B12 deficiency	957	14	282	8	1·95	0·025*	312	1	56	1	5·56	0·285	645	13	226	7	1·55	0·175
Congenital haemolytic anaemia	971	0	290	0			313	0	57	0			658	0	233	0		
Other haematological dis.	940	31	283	7	0·76	0·276	298	15	57	0	0·03	0·012*	642	16	226	7	1·25	0·558
Diabetes mellitus type 1	959	4	289	0	0·08	0·579	308	0	56	0			651	4	233	0	0·07	0·578
Crohn's dis.	960	3	286	3	3·36	0·140	308	0	55	1	61·51	0·154	652	3	231	2	1·91	0·611
Immuno-deficiency	946	17	274	15	3·05	0·000*	304	4	54	2	2·88	0·232	642	13	220	13	2·92	0·000*
Adrenal gland hypofunction	960	3	289	0	0·11	1·000	308	0	56	0			652	3	233	0	0·09	0·571
Diabetes mellitus type 2	953	10	285	4	1·36	0·913	303	5	54	2	2·31	0·294	650	5	231	2	1·16	1·000
Hypo-thyroidism	881	82	243	46	2·04	0·000*	304	4	56	0	0·13	1·000	577	78	187	46	1·82	0·000*
Hyper-thyroidism	952	11	285	4	1·23	0·669	308	0	55	1	61·51	0·154	644	11	230	3	0·78	0·434
Goiter	961	2	286	3	4·96	0·084*	308	0	56	0			653	2	230	3	4·19	0·116
Adrenal gland hyperfunction	962	1	289	0	0·30	1·000	308	0	56	0			654	1	233	0	0·26	1·000
Inborn metabolic dis.	958	5	288	1	0·72	1·000	306	2	55	1	2·91	0·395	652	3	233	0	0·09	0·571
Obesity	883	80	260	29	1·23	0·519	283	25	53	3	0·66	0·285	600	55	207	26	1·37	0·382
Hypoglycaemia	948	15	286	3	0·68	0·335	307	1	55	1	5·57	0·284	641	14	231	2	0·41	0·048*
Osteoporosis	948	15	279	10	2·27	0·010*	306	2	55	1	2·91	0·395	642	13	224	9	1·99	0·100
Delayed puberty	952	11	285	4	1·23	0·549	302	6	55	1	0·99	1·000	650	5	230	3	1·72	0·439
Precocious puberty	961	2	286	3	4·96	0·084*	307	1	56	0	0·50	1·000	654	1	230	3	8·01	0·058*
Amenorrhea	949	14	286	3	0·73	0·529	308	0	56	0			641	14	230	3	0·61	0·300
Pituitary gland dis.	961	2	287	2	3·35	0·230	307	1	55	1	5·57	0·284	654	1	232	1	2·82	0·456
Other metabolic dis.	936	27	282	7	0·87	0·569	299	9	56	0	0·06	0·365	637	18	226	7	1·11	0·857

Oesophageal cancer	998	0	298	0			314	0	59	0			684	0	239	0		
Stomach cancer	998	0	298	0			314	0	59	0			684	0	239	0		
Colon and rectal cancer	995	3	297	1	1·19	1·000	313	1	58	1	5·39	0·292	682	2	239	0	0·14	1·000
Liver cancer	998	0	297	1	36·95	0·230	314	0	58	1	59·47	0·158	684	0	239	0		
Lung cancer	997	1	297	1	3·36	0·407	313	1	58	1	5·39	0·292	684	0	239	0		
Melanoma, other skin cancers	996	2	297	1	1·76	0·544	314	0	59	0			682	2	238	1	1·50	1·000
Breast cancer	995	3	297	1	1·19	1·000	314	0	59	0			681	3	238	1	1·02	1·000
Cervical uterine precancerosis	936	13	271	7	1·87	0·062*	314	0	59	0			636	13	215	7	1·60	0·229
Cervical uterine cancer	990	8	292	6	2·55	0·012*	314	0	59	0			676	8	233	6	2·18	0·058*
Corpus uteri cancer	997	1	298	0	0·30	1·000	314	0	59	0			683	1	239	0	0·26	1·000
Ovarian cancer	997	1	298	0	0·30	1·000	314	0	59	0			683	1	239	0	0·26	1·000
Prostate cancer	998	0	298	0			314	0	59	0			684	0	239	0		
Lymphoma, myeloma multiple	997	1	298	0	0·30	1·000	313	1	59	0	0·48	1·000	684	0	239	0		
Leukaemia	998	0	297	1	36·95	0·230	314	0	58	1	59·47	0·158	684	0	239	0		
Bladder cancer	998	0	298	0			314	0	59	0			684	0	239	0		
Mouth, oropharynx cancers	998	0	298	0			314	0	59	0			684	0	239	0		
Neuroblastoma	998	0	298	0			314	0	59	0			684	0	239	0		
Adeno-carcinoma	998	0	298	0			314	0	59	0			684	0	239	0		
Papilloma cancer	998	0	296	2	70·79	0·053*	314	0	59	0			684	0	237	2	60·59	0·067*
Glioma, glioblastoma	997	1	298	0	0·30	1·000	313	1	59	0	0·48	1·000	684	0	239	0		
Other types of cancer	993	5	296	2	1·38	0·664	312	2	58	1	2·81	0·404	681	3	238	1	1·02	1·000
Urinary tract infections	761	188	197	81	1·66	0·000*	279	21	48	8	2·23	0·006*	482	167	149	73	1·41	0·003*
Nephrosis, glomerulonephritis	941	8	276	2	0·88	0·707	298	2	56	0	0·25	1·000	643	6	220	2	1·01	1·000
Bladder infection, cystitis	878	71	243	35	1·78	0·000*	296	4	55	1	1·44	0·577	582	67	188	34	1·57	0·003*
Prostate hypertrophy	944	5	277	1	0·73	1·000	295	5	55	1	1·16	1·000	649	0	222	0		
Gynaecological infections	777	172	220	58	1·19	0·077*	297	3	56	0	0·17	1·000	480	169	164	58	1·01	0·930
Obstetric complications	918	31	266	12	1·34	0·437	300	0	56	0			618	31	210	12	1·14	0·783
Recurrent abortion	933	16	267	11	2·41	0·002*	300	0	56	0			633	16	211	11	2·07	0·031*
Kidney stones	932	17	271	7	1·43	0·420	294	6	56	0	0·09	0·595	638	11	215	7	1·90	0·103
Other genitouranary dis.	930	19	272	6	1·09	0·803	295	5	53	3	3·38	0·116	635	14	219	3	0·64	0·266
Glaucoma	959	8	286	5	2·11	0·065*	308	0	57	1	59·35	0·158	651	8	229	4	1·44	0·506
Cataracts, clouding of the lens	962	5	290	1	0·72	1·000	306	2	58	0	0·25	1·000	656	3	232	1	1·00	1·000
Refractive errors	581	386	165	126	1·15	0·173	200	108	33	25	1·40	0·080	381	278	132	101	1·05	0·774
Hearing loss	925	42	274	17	1·37	0·195	295	13	52	6	2·64	0·004*	630	29	222	11	1·08	0·951
Macular degeneration	957	10	289	2	0·69	0·333	304	4	56	2	2·78	0·243	653	6	233	0	0·05	0·349
Strabismus	945	22	286	5	0·76	0·358	301	7	56	2	1·59	0·639	644	15	230	3	0·57	0·148
Sense of smell problems	944	23	284	7	1·02	0·844	297	11	57	1	0·52	0·279	647	12	227	6	1·44	0·386
Sense of taste problems	961	6	291	0	0·05	0·346	305	3	58	0	0·17	1·000	656	3	233	0	0·09	0·571
Sense of touch problems	963	4	288	3	2·53	0·205	307	1	57	1	5·38	0·292	656	3	231	2	1·92	0·610
Ringing in the ears	906	61	267	24	1·34	0·205	284	24	49	9	2·18	0·005*	622	37	218	15	1·16	0·783

Table 3. (*Cont.*)

Disorder/disease	Both genders						Men						Women					
	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis−	Toxo+ Dis+	OR	*p* value	Toxo− Dis−	Toxo− Dis+	Toxo+ Dis-	Toxo+ Dis+	OR	*P* value
Sense of motion problems	935	32	274	17	1·82	0·008*	305	3	56	2	3·68	0·180	630	29	218	15	1·50	0·144
Congenital unilat. blindness	967	0	291	0			308	0	58	0			659	0	233	0		
Amblyopia, lazy eye	922	45	277	14	1·04	0·973	297	11	55	3	1·51	0·383	625	34	222	11	0·92	0·560
Other sense organ dis.	929	38	278	13	1·15	0·488	291	17	56	2	0·64	0·329	638	21	222	11	1·51	0·106
Cranial nerves neuropathy	972	1	292	1	3·33	0·409	308	1	57	0	0·49	1·000	664	0	235	1	31·07	0·262
Extremity neuropathy	960	13	288	5	1·30	0·686	305	4	57	0	0·13	1·000	655	9	231	5	1·59	0·345
Demyelination	968	5	292	1	0·71	1·000	309	0	57	0			659	5	235	1	0·60	1·000
Stroke	971	2	293	0	0·16	1·000	308	1	57	0	0·49	1·000	663	1	236	0	0·26	1·000
Multiple sclerosis	968	5	290	3	2·03	0·396	308	1	56	1	5·49	0·288	660	4	234	2	1·44	0·656
Epilepsy	967	6	287	6	3·37	0·001*	306	3	57	0	0·17	1·000	661	3	230	6	5·65	0·012*
Migraine	747	226	210	83	1·31	0·005*	269	40	44	13	1·99	0·004*	478	186	166	70	1·08	0·434
Stuttering	960	13	289	4	1·04	0·818	303	6	56	1	0·97	1·000	657	7	233	3	1·23	0·527
Tics	944	29	271	22	2·64	0·000*	298	11	53	4	2·07	0·070	646	18	218	18	2·96	0·000*
Muscle twitch, fasciculation	910	63	264	29	1·59	0·002*	298	11	48	9	5·08	0·000*	612	52	216	20	1·09	0·554
Cramps	892	81	264	29	1·21	0·199	292	17	50	7	2·42	0·005*	600	64	214	22	0·97	0·829
Other neurological dis.	957	16	282	11	2·34	0·002*	305	4	54	3	4·26	0·079	652	12	228	8	1·91	0·059*
Unipolar depressive dis.	924	39	285	8	0·67	0·109	300	4	56	2	2·74	0·247	624	35	229	6	0·47	0·006*
Bipolar disorder	948	15	289	4	0·89	0·809	301	3	57	1	1·87	0·504	647	12	232	3	0·71	0·408
Schizophrenia	960	3	291	2	2·23	0·332	303	1	58	0	0·47	1·000	657	2	233	2	2·82	0·283
Anxiety dis.	905	58	275	18	1·02	0·699	295	9	53	5	3·11	0·002*	610	49	222	13	0·73	0·215
Alcohol use disorder	951	12	293	0	0·03	0·003*	299	5	58	0	0·10	1·000	652	7	235	0	0·04	0·199
Gambling	961	2	292	1	1·72	0·550	302	2	57	1	2·77	0·409	659	0	235	0		
Parkinson's disorder	962	1	293	0	0·30	1·000	303	1	58	0	0·47	1·000	659	0	235	0		
Alzheimer', other dementias	963	0	293	0			304	0	58	0			659	0	235	0		
Drug use dis.	955	8	289	4	1·67	0·220	301	3	58	0	0·17	1·000	654	5	231	4	2·28	0·253
Post-traumatic stress dis.	949	14	286	7	1·67	0·135	300	4	58	0	0·13	1·000	649	10	228	7	2·00	0·059*
Obsessive compulsive dis.	943	20	290	3	0·50	0·126	298	6	57	1	0·94	1·000	645	14	233	2	0·41	0·097
Panic disorder	934	29	285	8	0·91	0·717	301	3	54	4	7·36	0·014*	633	26	231	4	0·43	0·011*
Insomnia, primary	869	94	259	34	1·22	0·157	288	16	53	5	1·72	0·135	581	78	206	29	1·05	0·800
Learning disability	931	32	270	23	2·48	0·000*	294	10	53	5	2·80	0·005*	637	22	217	18	2·40	0·000*
Borderline personality dis.	954	9	291	2	0·76	0·639	300	4	58	0	0·13	1·000	654	5	233	2	1·16	1·000
Antisocial personality dis.	958	5	290	3	2·01	0·399	301	3	56	2	3·64	0·183	657	2	234	1	1·47	1·000

Attention deficit, hyperactivity	938	25	285	8	1·06	0·694	297	7	54	4	3·17	0·005*	641	18	231	4	0·63	0·283
Tourette's syndrome	963	0	293	0			304	0	58	0			659	0	235	0		
Autism	928	26	276	14	1·82	0·008*	286	13	47	8	3·76	0·000*	642	13	229	6	1·31	0·470
Other mental health dis.	930	33	278	15	1·53	0·034*	293	11	55	3	1·49	0·401	637	22	223	12	1·56	0·057*
Erectile dysfunction	941	31	285	10	1·07	0·742	277	31	48	10	1·87	0·016*	664	0	237	0		
Too low sex appetency	775	197	222	73	1·29	0·026*	268	40	52	6	0·78	0·405	507	157	170	67	1·27	0·076
Too high sex appetency	915	57	282	13	0·74	0·151	269	39	54	4	0·52	0·062	646	18	228	9	1·42	0·121
Too low sex potency	966	6	294	1	0·59	1·000	303	5	57	1	1·14	1·000	663	1	237	0	0·25	1·000
Quality of sex	882	90	262	33	1·24	0·177	282	26	56	2	0·40	0·050	600	64	206	31	1·41	0·041*
Paraphilias (severe)	971	1	294	1	3·30	0·412	307	1	58	0	0·48	1·000	664	0	236	1	30·94	0·263
Paraphilias (mild)	1142	11	332	1	0·34	0·092*	357	8	69	0	0·06	0·366	785	3	263	1	1·06	1·000
Other sex related dis.	937	35	282	13	1·24	0·308	291	17	53	5	1·64	0·173	646	18	229	8	1·26	0·469
Spondylosis, spondylitis	951	4	276	4	3·44	0·083*	298	2	54	0	0·26	1·000	653	2	222	4	5·74	0·041*
Myopathy	953	2	280	0	0·16	1·000	300	0	54	0			653	2	226	0	0·14	1·000
Backbone pain	635	320	166	114	1·36	0·005*	222	78	38	16	1·20	0·400	413	242	128	98	1·31	0·049*
Osteoporosis	943	12	272	8	2·32	0·021*	299	1	53	1	5·63	0·282	644	11	219	7	1·88	0·247
Rheumatoid arthritis, inflam.	934	21	273	7	1·15	0·949	294	6	53	1	1·00	1·000	640	15	220	6	1·17	0·943
Rheumatic fever	953	2	278	2	3·43	0·223	298	2	54	0	0·26	1·000	655	0	224	2	61·39	0·066*
Psoriatic arthritis, arthropathy	954	1	280	0	0·31	1·000	300	0	54	0			654	1	226	0	0·26	1·000
Scoliosis	812	143	214	66	1·75	0·000*	266	34	39	15	3·01	0·000*	546	109	175	51	1·46	0·001*
Scheuermann's disease	942	13	274	6	1·60	0·218	292	8	52	2	1·45	0·527	650	5	222	4	2·35	0·246
Osteoarthrosis	923	32	265	15	1·64	0·091*	290	10	49	5	2·98	0·003*	633	22	216	10	1·34	0·728
Other musculoskeletal dis.	928	27	269	11	1·41	0·132	291	9	52	2	1·29	0·682	637	18	217	9	1·48	0·110
Bronchitis	888	67	250	30	1·59	0·008*	285	15	49	5	1·96	0·061	603	52	201	25	1·44	0·088
Asthma	854	101	233	47	1·71	0·000*	270	30	45	9	1·81	0·031*	584	71	188	38	1·66	0·000*
Recurrent respiratory infect.	870	85	248	32	1·32	0·098*	276	24	52	2	0·46	0·096	594	61	196	30	1·49	0·027*
Other respiratory dis.	929	26	265	15	2·03	0·002*	288	12	51	3	1·44	0·435	641	14	214	12	2·57	0·000*

Numbers of *Toxoplasma*-free subjects without particular disorders, *Toxoplasma*-free subjects with particular disorders, *Toxoplasma*-infected subjects without particular disorders, *Toxoplasma*-infected subjects with particular disorders, odds ratios (OR), and statistical significance (*P*), respectively, are shown in six columns of each section. The effect of age on health status was controlled in partial Kendall's correlation (bivariate non-parametric) test when the incidence of particular disorder was higher than 9, otherwise the univariate exact Fisher test was used for computing statistical significance. ORs higher than 1 indicate positive association of *Toxoplasma* infection with incidence of particular disorder. Asterisks indicate results significant in two-sided tests after Benjamini-Hochberg procedure correction for multiple tests. *P*-values <0·0005 are coded as 0·000.

disorders and seroprevalence of toxoplasmosis in particular countries (Flegr *et al*. 2014). Many of the observed associations have been already reported to exist in various, mostly small-scale, case-control studies, for review see (Flegr *et al*. 2014). Some of the observed associations were, however, new or at least underreported. For example, the strong associations of toxoplasmosis with many digestive system disorders (acute and chronic diarrhoea, constipation, flatulence, chronic gastritis, maldigestion and food intolerance, weight loss, pyrosis and reflux, gall bladder attacks and coeliac disease) suggest that namely the digestive organs of infected hosts are affected by toxoplasmosis. Being a food-borne parasite, *T. gondii* first comes into contact with the host's intestinal lumen and cells, including enterocytes, fibroblasts and intestinal neurons. The intestine is not only the first site of infection but also a site where the first line of immune response usually occurs. As a result of the immune system's efforts to fight off the infection, cytokine levels in the nearby tissue are altered. This could result in acute inflammation and immunization of the host by various food antigens and autoimmunization. All these could be the causes of the observed toxoplasmosis-associated gastrointestinal afflictions (Prandota, 2012; Bhadra *et al*. 2013).

Another class of toxoplasmosis-associated health problems that had been underreported in the scientific literature were immunity-related problems. The general pattern was that infected subjects expressed symptoms of immunodeficiency, allergy and also autoimmunity. Such changes could result in the observed increased risk of bronchitis, bacterial skin infections, urogenital infections, hypothyroidism, osteoporosis and scoliosis. Some of observed blood cell-related disorders, e.g. mononucleosis, could be the results of the manipulative activity of *Toxoplasma*, either the suppression or downregulation of the immune defence of the infected host (Flegr & Stříž, 2011) or enhancement of migratory activity of the infected leukocytes to facilitate transport of *Toxoplasma* to other organs, including the brain (Lambert *et al*. 2011; Fuks *et al*. 2012).

Very strong associations were also observed between toxoplasmosis and certain neurological problems, such as tics, fasciculations, migraine and headache. The association was found also with epilepsy, which was in an agreement with previous correlation and case control studies (Ngoungou *et al*. 2015). The relation between toxoplasmosis and neurological problems could be explained by the presence of tissue cysts of the parasite and associated lesions (Berenreiterova *et al*. 2011) in the infected brain tissue.

The present study showed the expected associations between toxoplasmosis and some neuropsychiatric disorders, for a review see (Flegr, 2013*a*). This concerned epilepsy, migraine, tics, fasciculation, autism, learning disabilities. Some associations, e.g. cramps, panic disorder, unipolar depression, anxiety disorder, attention deficit and hyperactivity were significant only in men and some were just borderline, e.g. posttraumatic disorder in women, probably due to the low number of the affected subjects in the study population or opposite effects of toxoplasmosis in men and women. In addition to the expected positive correlations, certain negative associations between toxoplasmosis and mental health disorders were observed. For example, a negative association with unipolar depression and panic disorder in women and a negative association with alcohol use disorder in both men and women. The lower incidence of alcohol use disorder could be the result of decreased novelty seeking observed in infected subjects (Flegr *et al*. 2003; Skallová *et al*. 2005). A lower incidence of panic disorder is in agreement with the results of earlier studies where infected women under imminent danger remained rather slow and passive and had a weak instinct for self-preservation – they claimed to stay abnormally calm in dangerous situations (Flegr, 2010, 2013*b*). No explanation was found for the observed lower incidence of unipolar depression in the infected women – six of 235 (2·6%) *vs* 35 of 659 (5·3%) in *Toxoplasma*-free women (OR = 0·47, $P = 0·006$). A positive rather than negative association should be expected in view of the decreased concentration of tryptophan (Hsu *et al*. 2014) as well as in view of the increased incidence of suicides in the infected subjects (Pedersen *et al*. 2012; Hsu *et al*. 2014). The absence of association between toxoplasmosis and unipolar depression in the mixed population of men and women was in agreement with the negative results of some previous studies (Gale *et al*. 2014; Markovitz *et al*. 2015).

Study limitations

The design of the present study, a cross-sectional study performed on a large cohort of internet users, has some strengths and some limitations. It is in principle the only method suitable for the assessment of the real strength of association between toxoplasmosis and other disorders, and therefore the real potential health impact of toxoplasmosis in the general population. It must be emphasized, however, that not all observed associations exist due to the effect of toxoplasmosis on human health, see the discussion about the causality problem below. Also, the results concerning the associations of an environmental factor, here a past *Toxoplasma* infection, with the incidence of uncommon diseases are not reliable enough because of low number of subjects with rare diseases in our population sample.

A second limitation of the present study is the possible influence of a strong sieve effect. The

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respondents entered the study voluntarily, without any financial reward. It is highly probable that only a certain kind of people (e.g. extreme altruists) are willing to spend 30–60 min to answer the questions of the electronic questionnaire. The Facebook community Guinea Pigs consists primarily of individuals interested in participating in evolutionary psychology studies (Flegr & Hodny, 2016). However, this test was advertised as part of a study of the influence of the blood group (not toxoplasmosis) on human performance and health. It is, therefore, possible that people with special interest in their own health, e.g. the people with health problem, preferably took part in the present study. This could result in positively biased incidence rates of particular disorders, therefore this part of our results cannot by generalized on whole Czech population. However, there is no reason to expect that such a bias will differ between *Toxoplasma*-infected and *Toxoplasma*-free subjects.

The study was based on the information provided by the subjects themselves and not on objective medical records. It is highly probable that some part of the data is wrong or at least obsolete. For example, some of the subjects who tested negative for toxoplasmosis in the past could have acquired the infection in the meantime. Again, such errors increase the risk of false negative and not false positive results of statistical tests (Flegr, 2016).

All participants of the present study have been informed about their toxoplasmosis status in the past, mostly after finishing our previous behavioural studies. It is possible that the awareness about being infected by *Toxoplasma* could have a negative effect on the subjects' health or that it motivates the infected subjects to have more medical examinations, which could reveal more health disorders. However, up to now, all medical textbooks say, and nearly all medical doctors firmly believe, that latent toxoplasmosis, i.e. the presence of low concentration of anamnestic anti-*Toxoplasma* IgG antibodies have no impact on the health and quality of life of a subject. Participants of our past studies have obtained this information (in a written form) as part of debriefing after finishing their participation in (double blind) behavioural experiments. Therefore, it is rather improbable that the knowledge of their latent infection could have a strong impact on their health or on the frequencies or intensity of their medical examinations. Moreover, some of the observed associations between toxoplasmosis and various disorders have already been described in literature, mostly on the basis of case-controls studies. In these studies, no effect of the participants' knowledge of toxoplasmosis status exists. Still it would be very important to repeat our cohort study on a similar population of subjects who will be tested for the presence of anti-*Toxoplasma* antibodies after completing their anamnestic questionnaire.

It must be emphasized that no observational study could definitively solve the causality problem. The cross-sectional study, including our cohort study, could quantify the probability that statistical association exists between the effects A and B. However, it cannot decide whether A (e.g. toxoplasmosis) is the effect of B (impaired health status, for example impaired immunocompetence) or whether it is the cause the B. There is a relatively low probability that, for example, schizophrenia, muscular fasciculation or pneumonia could cause the *Toxoplasma* infection. It is, however, always possible that both A and B are caused by an unknown factor C, for example by being born in a village, rather than in a large city. It is often believed that the problem of causality could be solved by longitudinal studies. However, even this is only partly true in the real word. It is possible that the specific symptoms of a disorder, e.g. acquired immunodeficiency, evolve earlier than the humoral anti-*Toxoplasma* immunity and therefore the disorder could be diagnosed earlier than the presence of *Toxoplasma* infection in some affected subjects. In the other words, even the criterion of temporality could fail. Therefore, any conclusions regarding the causal relation between two factors based on the results of observational study must always be considered just preliminary, and must be confirmed by manipulative study, for example by the experimental infection of laboratory animals.

This study is of exploratory nature. Still, the results in tables are presented after the correction for multiple tests. It must be stressed out, however, that the number of the obtained significant results is ten times higher than the theoretical number of false significant results obtained due to multiple tests. This suggests that most of the positive results reflect the existence of real biological effects rather than being an artefact of the multiple tests.

In conclusion, our study demonstrated a very strong negative association between toxoplasmosis and the health status of infected subjects. The responders diagnosed in the past with toxoplasmosis on the basis of presence of anti-*Toxoplasma* antibodies expressed a higher incidence of many, but not all, disorders, suggesting that the association with toxoplasmosis is not only strong, but also specific. Unlike the previous case-control studies and anecdotal observations, this explorative non-clinical cohort based study suggests that the immune system and digestive organs could be most strongly affected by the infection.

Toxoplasma is probably the most common parasitic disease in developed countries. Its prevalence is declining in the USA and in most countries in Europe. However, it is on the rise in highly populated Asian countries like China and Korea. Therefore, the global impact of toxoplasmosis is

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probably increasing. Neither an effective method for the treatment of latent toxoplasmosis nor an effective human vaccine is now available. The results of recent studies, including the present one, suggest that searching for effective drugs and a safe vaccine is of utmost importance.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0031182016001785.

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