Author's response to reviews

Title: Longer pregnancy and slower fetal development in women with latent "asymptomatic" toxoplasmosis

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Author's response to reviews: see over
Dear Editors

Please find enclosed a corrected electronic version of our manuscript entitled “ Longer pregnancy and slower fetal development in women with latent “asymptomatic” toxoplasmosis” (originally “Longer gravidity and the slower development of the foetus in women with latent “asymptomatic” toxoplasmosis”).

We have performed all changes recommended by the reviewer’s comments (see the List of changes). We appreciate the helpful suggestions of the reviewer, which allowed us to improve the quality of our manuscript. We hope that you find this new version suitable for publishing in BMC Infectious Diseases.

Sincerely Yours

Jaroslav Flegr

List of major changes

1)  The methods are clearly stated but incompletely reported. Last menstrual period (LMP) is essential information in the analysis. It is expected that at least 20% of the pregnant population has no certain account of LMP, or the LMP may be irregular. The present study has no account of menstrual data. Irregular LMP is also more common with growing age and thus a possible confounder (in the present study maternal age was an important factor).

   We are aware of the problems with LMP (irregularity, poor or missing records for some women, etc.). However, as both age (and sometimes also LMP) were included into our analyses, the risk of systematic error due to the confounding effect of LMP is very low. Of
course, the low quality of LMP data can increase the risk of Type II error (i.e. false negative results). In the same time, it cannot increase the risk of Type I error (of false positive results).

We have admitted possible problems in the Methods section of the corrected version of the manuscript:
“The data on the last menstruation can be affected by stochastic errors which are not likely to be responsible for false positive results.”

2) During the period 1996-2004 women may have given birth to more than one child, and as mentioned below, possibly more frequently repeat male births. I cannot see that siblings are accounted for.

Data for all twins were excluded from the data set. We considered every birth to be an independent event (all women changed their age, parity status, some of them sex of child and some even the toxo status). Of course, in a very strict sense, the repeated births by the same women can be considered by some authors as pseudoreplications. On the other hand, in the current demographic situation in the Czech Republic (1.28 child/woman), less than 10% of women could have been included into our file more than once. We are unable to identify such women in our anonymised data set. However, our results were rather robust and all relevant effects would most probably remain significant even if the correction of d.f. for 10-20% pseudoreplications was (could be) done (checked).

3) I would expect an increased incidence of toxoplasma sero-conversion, multiparity, and possibly also increased number of male neonates (if the society give preference to repeat male rather than female births) with growing age. I would like to see this being controlled for in the statistics.

The possible confounding variable age was included in all analysed models. (We checked Dr. Kiserud’s suggestions: The number of males did not increase with age in our experimental set, possibly (at least partly) because of the alarming demographic situation in the Czech Republic.)
4) Birth (and pregnancy duration) is not normally distributed, which need to be taken into account during the analysis also in the present study.

The pregnancy duration (UZ, PM) had a normal distribution. Moreover, GLM tests (in particular, with large data sets) are considered very robust with respect to data normality.

We included the following information into the corrected version of the manuscript:

“All statistical testing including the evaluation of statistical test assumptions (normality of data distribution, normality of residuals and homogeneity of variances) were done using the Statistica® 6.0”

5) A more detailed account of the toxoplasma test is required (cut off level for pregnant women, test performance etc).

All sera from clients of the private gynecology laboratory Gest are screened for toxoplasmosis in the Diagnostic Laboratory of the Central Military Hospital, Prague, using a highly sensitive indirect immunofluorescence test.

In the corrected version of manuscript we changed

“The presence of anamnestic antibodies against Toxoplasma was diagnosed with the indirect immunofluorescence test (IIFT).”

to

“The presence of anamnestic antibodies against Toxoplasma was diagnosed with the indirect immunofluorescence test (IIFT) at dilutions between 1:8 and 1:1024. The samples with specific fluorescence visible in a 1:16 or higher dilution was considered as Toxoplasma-positive.”

6) There were notably more male than female neonates of sero-positive mothers. Have the authors searched for information that the toxoplasma serology may be different in male and female pregnancies, which possibly would require a different cut off levels for the test?
No information is available on possible influence of the male/female neonate on maternal serology. However, the influence of latent *Toxoplasma* infection on the probability of birth of a son was reported not only in a large observational study performed on 1803 infants (Naturwissenschaften 94: 122-127, 2007) but was also recently confirmed by experimental infection in laboratory mice (in press).

7) *Information on when ultrasound measurements were taken is contradictory, 8-12, 16, 20 or 30 weeks or all (page 5), and the results of these measurements are not displayed, nor are the results of the present analysis of these data.*

The complete bioparameters data are available only for pregnancy weeks 20 and 30. The Gynaecologist usually uses the data from the first ultrasonography (performed at pregnancy week 8-12) and from that at pregnancy week 16 for the computation of the estimated pregnancy duration but does not enter them into the medical records.

We added a new table with the results of analyses of the bioparameter data into the corrected version of the manuscript:

Table 1: Fetal ultrasonography data in *Toxoplasma*-positive and *Toxoplasma*-negative women at pregnancy weeks 20 and 30. Fetal parameters are shown in millimetres. The results of statistical tests (F and *P*) were obtained with toxoplasmosis as the independent variable and maternal age and pregnancy length estimated from the date of the last menstruation as continuous predictors. BPD – biparietal diameter, AC – abdominal circumference, FL – femur length.

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<thead>
<tr>
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<th>Pregnancy week 20</th>
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<th>Pregnancy week 30</th>
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<td></td>
<td>BPD</td>
<td>AC</td>
<td>FL</td>
<td>BPD</td>
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<tr>
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<td>(toxo.neg./toxo.poz.)</td>
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<td>(toxo.neg./toxo.poz.)</td>
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<tr>
<td>952</td>
<td>(760/192)</td>
<td>925</td>
<td>(378/187)</td>
<td>952</td>
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<tr>
<td><em>F</em></td>
<td>0.0</td>
<td>1.4</td>
<td>3.1</td>
<td>0.2</td>
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</table>
8) Gender is an important factor in growth and also in length of pregnancy. Boys tend to be assigned to an overestimated gestational age by ultrasound biometry (typically 1.5 days), tend to grow faster, and have shorter pregnancies according to LMP (linked to paternal birthweight). The analysis and results are presented such that I cannot appreciate whether such effects have been taken into consideration, or how effects are translated into differences in days.

We are aware of the difference in the fetal growth rate between males and females. In fact, this was the basis of our hypothesis No. 4. This hypothesis was rejected based on the positive results of analyses performed separately for the male and female newborns.

9) There is no information in the manuscript that permits controlling for social factors or other infectious conditions that may be more common in the toxoplasma seroconverted part of the population. If not available, the point should be discussed in the discussion section with references made to the literature. I expect that the discussion, conclusion and abstract may be rephrased according to a renewed analysis.

We included the following paragraph into the corrected version of the manuscript:

“It cannot be excluded by any case-control study, however, that Toxoplasma infection is in fact only statistically associated with some unknown socioeconomic or possibly even infection factor that is responsible for the observed effects. On the other hand, most toxoplasmosis-associated effects such as changes in novelty seeking, activity, reaction times and the secondary sex ratio [19] have been already confirmed by experimental infection of laboratory mice or rats.
Therefore, the direct effects of *Toxoplasma* on the organism of its host seem to be the most parsimonious explanation for the observed phenomena.”

10) *The language needs attention*

The corrected version of the manuscript was extensively edited by a professional editor.