Author's response to reviews

Title: Association between congenital toxoplasmosis and parent-reported developmental outcomes, concerns, and impairments, in 3 year old children.

Authors:

K Freeman (kfreeman@montefiore.org)
A Salt (a.salt@ich.ucl.ac.uk)
A Prusa (arprus@gmail.com)
G Malm (gunilla.malm@hs.se)
N Ferret (ferret.n@chu-nice.fr)
W Buffalono (wilma@cds.unina.it)
D Schmidt (DRS@ssi.dk)
Hk Tan (h.tan@ich.ucl.ac.uk)
Re Gilbert (r.gilbert@ich.ucl.ac.uk)

Version: 3  Date: 7 April 2005

Author's response to reviews: see over
Editor
BMC Pediatrics

7 April 2005

Dear Sir or Madam

Association between congenital toxoplasmosis and parent-reported developmental outcomes, concerns, and impairments, in 3 year old children

Thank you for sending us the helpful and constructive referees’ comments. We enclose a revised manuscript in which we have endeavoured to address all of their comments. Our changes are marked in blue to make it easy for you to check them. Please revert to no highlighting afterwards but if you want me to send an un-highlighted version please let me know. Below, we outline in detail, how we have responded to each of the comments.

We look forward to hearing from you in the near future.
Yours sincerely,

Ruth Gilbert
Responses to Reviewers’ Comments

Reviewer 1. Gary Holland

1. Comment: Clarify extent to which questionnaire has been validated
   - Response: We discuss the validation of the questionnaire in detail in para 4 or the discussion. We have added a statement to the methods (p4) to explain that the questionnaire as a whole has not been validated but is composed of previously validated instruments in whole or part.

2. Measures for assessing visual loss are not sensitive enough. Results might differ if they assess loss of vision despite glasses or strabismus surgery.
   - Response. Our question about visual impairment did specify ‘with glasses if usually worn’ (see question 19 in questionnaire and footnote to table 4). However, we had no information on strabismus surgery though this is likely to be too rare to affect the results. Table A3 supplied as an appendix for the reviewers shows that 11 children in total had strabismus (referred to as lazy eye). We accept that more sensitive measures could be used to measure impairment but even using parental responses about blind or limited vision while wearing glasses, we found a significant difference (table 4).

3. Discuss low response rates for vision and how this might affect the results
   - Response: The reviewer is correct that the response to the vision question was 92% compared with 99% response for many other questions. See last para of results (in paper on Determinants of response). We have added a sentence to the discussion to the effect that under-reporting of visual impairment in uninfected children may attenuate the association of congenital toxoplasmosis with visual impairment.

4. Discuss why questionnaires were returned at different ages.
   - Response: The age at return of questionnaires is one of the outcomes examined in the second paper (Determinants of response…). We feel that it would duplicate that paper to report and discuss results here. As all analyses are adjusted for age at response, we have taken the variation in response age into account

5. Is maternal age expected to be associated with development outcome?
   - Response: This was an hypothesis that we tested in the analysis. However, to avoid referencing other work in the midst of the results, we have deleted the word ‘expected’.

6. Why would outcomes vary by site? Is this an artifact?
   - Response: We discuss why outcomes might vary by site in the first sentence under ‘analyses’ in the methods. In addition, the last paragraph of the discussion goes into some detail about the reasons why parental anxiety might vary by site.

7. Define key terms like behavior abnormalities
   - Response. The type of function measured is best described by examining the questionnaire, from which we derived an interval score. Referral to
definitions based on other instruments could be misleading. We have also clarified how abnormality is defined in the methods (see outcome). Fuller details are given in the accompanying paper, which we hope will be published alongside this paper.

8. Lack of relationship between serious problems before 4 months should be discussed.
   • Response. The sentence referred to in para 3 of the discussion is about the response to the questionnaire, not evidence of abnormality based on the answers given in the questionnaire. We accept that this can be confusing. The sentences have been changed in the discussion.

9. Typographical error.
   • Response. Corrected

10. Discuss the fact that 21 fetal terminations were not included. Discrepancy between 17 and 21 not followed up.
    1. Response: We have included a sentence in the methods (3rd para under ‘clinical follow up’) that clarifies that 21 were lost to follow up, but 4 of these died (hence the confusion with 17).

11. Correct duplicate references
    • Response: references checked and corrected

12. Compare clinician findings with parental reports
    • Response: As mentioned in the discussion, this will be the subject of another report. However, for vision, we do report clinician findings for the infected children in the 5th paragraph of the discussion

**Reviewer 2: Jeffery Jones**

1. Abstract Add word treated
   • Response. The population includes children of treated and untreated pregnant women, which is why it is a unique cohort. We have explored possible effects of prenatal treatment in the paper. Hence to imply that all are treated would be inappropriate.

2. Conclusions: Add words
   • Response: We have added ‘at 3 years of age’ to 1st sentence under Conclusions

**Methods:**

3. Were uninfected control children with significant problems excluded?
   • Response. The control population comprised children born to toxoplasma infected women who did not develop congenital infection. Both infected and uninfected populations included children with impairment due to other reasons (eg prematurity). If such children had been excluded from the control population and not the infected population this would have been a source of bias, making congenital toxoplasmosis appear more harmful.
Our design controls for many of the maternal factors affecting acquisition of maternal infection.

**Analysis**

4. p. 7 last para: cut-off
   - Response. Typo now corrected.

5. p. 8 Were results similar if one SD below the mean was used.
   - Response. We modelled the outcome as a continuous ranked variable, as well as a dichotomous variable with a cut-off equivalent to the 10% with the lowest score in the uninfected population. Aas reported in the results, the results did not differ with either method. We have not explored a cut-off of 1 SD for two reasons. First, imposing different cutpoints may lead to increased false positive findings—we wanted to avoid that. Second, our aim was to detect moderate to severe abnormalities. The limitations of parent questionnaires for detecting mild abnormalities is well recognized.

**Results**

6. Page 10, para 1: Parents with uninfected children with impairments might be more likely to respond.
   - Response. To deal with this problem we performed the sensitivity analysis comparing infected and uninfected children with no abnormalities by 4 months. The only factors that were associated with increased response were 1) direct involvement in follow up and access to an address register, and 2) infection with congenital toxoplasmosis. We had limited power to assess the effect of presence/absence of severe neurologic problems within the first 4 months. Examination of the association between early clinician findings and subsequent development will be the subject of a further report confined to infected children and using more examination data than is available for the infected vs uninfected comparison.

**Discussion**

7. Page 15, last para: 17 terminations is a real limitation of the study and should be mentioned in the abstract..
   - Response: We accept that the fact that some terminations were performed affects the generalisability of the study to settings where terminations are not performed. Hence, we consider it appropriate to discuss this point in the discussion (penultimate paragraph). Only 8 terminations were performed after a positive fetal diagnosis and only 4 of these had evidence of intracranial or disseminated infection. Hence, if these children had survived to 3 years, their inclusion is unlikely to have substantially altered the results.

**Conclusions**

8. Include at end of first sentence “by 3 years of age”
   - Response: phrase included.

**Discretionary**

9. Page 7 para 1. A neuropsychiatric evaluation would be more sensitive.
• Response: We make the point that further research is required involving more detailed neurological assessments in paragraph 5 of the discussion.

10. Comment. The study should be continued into the early school years.
• Response: In the conclusion we make the point that more subtle differences between infected and uninfected children may become apparent later in childhood.

11. Were some uninfected children subsequently infected after birth and developed eye lesions as a result?
• Response: Acquisition of toxoplasma infection after infancy was not monitored in the uninfected cohort. However, the possibility of retinchoroiditis due to postnatally acquired toxoplasmosis is extremely small. Precise data on this risk are lacking but we can assume an upper estimate of the incidence of infection of about 1% between 1 and 3 years, and a risk of ocular lesions in the year after infection at the most of 0.5%. ie. Absolute risk would be 5/100,000

Reviewer 3: Paul Hewson

Major Compulsory Revisions
1. Sentence not complete in analysis section.
• Response: Typing error corrected.

2. Give references to indicate exactly what models are being used.
• Response: Regarding the statistical analysis, the generalized linear model was used for France to better explore differences among the several centers nested within France. No other country providing survey data had multiple centers. We have added a reference to the model used to the methods (para 2 of analyses) [Agresti, A. (2002), Categorical Data Analysis(2nd Edition), New York: John Wiley & Sons, Inc. Chapter 5, pp. 143-145.

3. Give more details on the models used, how they were applied and how well they fit.
• Response: A generalized estimating equation (SAS Version 9.1 PROC GENMOD with the ASSESS options to assess fit of the model) with centers nested within country was derived to determine characteristics associated with each dichotomized outcome. This description has been added to the methods (analysis, paragraph 2.

• Regarding the fit of the generalized linear models, the only model that didn’t fit well was that for cognition. Deviance statistics and Pearson $\chi^2$ divided by its degrees of freedom indicated lack of overdispersion of the models, since values were close to 1.

4. More clarity needed on how the odds ratios were derived.
• Response: Odds ratios were derived by exponentiating parameter estimates, as well as lower and upper bounds for corresponding 95%
confidence intervals. For logistic regression. Wald estimates were used. Sentence now added to the methods (analyses, para 2).

5. What is the rationale of using a p value of 0.2 for inclusion of confounders?
   - Response: The choice of a cutoff for potential confounders of p<.20 was based on the modeling recommendations set forth in DW Hosmer, SI Lemeshow. Applied Logistic Regression (2000) John Wiley and Sons, pp 82-89. Chapter 4 “Model-Building Strategies and Methods for Logistic Regression”. Although they suggested using a p-value cutpoint of .25, because center variables were included in each model to assess the additional effect of the covariate, and because of the limited sample size, .20 was thought to be appropriate. This reference has been added to the methods.

6. Why should the significance of maternal age not be anything more than an event due to multiple comparisons?
   - Response: We agree that the issue of multiple outcomes is important. Meinert states that “this is the most difficult to address. The usual approach is to ignore the interdependence and to make comparison involving the different outcome measures as if they were independent of one another.” Meinert CL. Clinical Trials: Design, Conduct and Analysis (1986). Oxford University Press. We have emphasized the multiple endpoint problem in the discussion (paragraph 2) which can be due to multiple analyses of different and perhaps correlated endpoints resulting in inflation of type I error, producing false positive findings. We have emphasized caution in interpreting the results elsewhere in the discussion.

7. Is the primary outcome of the study really parental anxiety?
   - Response: We have clarified what the primary outcomes were for the study in paragraph 2 of the ‘outcomes’ section of the methods.

9. How was the sample size arrived at?
   - Response: Explanation of sample size calculation added as last paragraph in the methods.

Minor essential revisions

8. To whom might the full assessment tools have been unacceptable? What evidence is there to support this statement?
   - Response: We have clarified that a questionnaire that took a long time to complete might be unacceptable to parents (sentence amended). We have added a reference to a systematic review that shows that long questionnaires are less likely to be completed.

9. How much more difficult might an assessment at school entry have been? Is it possible to estimate the possible additional losses to the study in delaying the assessment?
   - Response: We agree that an assessment at school entry is desirable but has not proved possible. Although we have a detailed protocol developed and piloted for this purpose, including clinician assessments, the study has not been funded.