Reviewer's report

Title: The dynamics of nasopharyngeal streptococcus pneumoniae carriage among rural Gambian mother-infant pairs

Version: 2 Date: 5 April 2010

Reviewer: Simo Granat

Reviewer's report:

The original review, as well as this re-review, was carried out together with Dr Kari Auranen who focused particularly on reviewing the statistical part of the manuscript. As advised by the editor, this was mentioned in the original review in the confidential section of the review. We have now carefully read and considered the authors' comments to our review and re-reviewed the edited version of the manuscript.

Major compulsory revisions

Permutation test

1. We find the authors' research question about describing the number of serotypes the child carries valid and important. We appreciate the clarifications regarding the statistical (permutation) test procedure to compare the observed and expected distributions of the number of serotypes 'ever carried' by the child. The principal aim of our previous comments was not to criticise the test per se but ask the authors to be more specific on how the test statistic was actually constructed. Although the response we now received makes the construction more clear than before, we have to repeat some of the specific queries.

2. The core problem is that in the permutation test an assumption is made that in the study set-up the observed carriage of a serotype is independent of observed carriage of another serotype. In reality, due to the microbiological method employed (see below), the observed carriage of different serotypes cannot be dealt with independently.

3. As for co-colonisation observed in the data, the (microbiological) methodological clarification makes it clear that it was possible to find more than one serotype per sample. However, culturing and serotyping four colonies from a sample is grossly inadequate to estimate the true level of co-colonisation, and this method is really more apt to detect the dominant serotype in the sample, though finding multiple serotypes by chance is of course possible.

4. The way the permutation test is now carried out, by assigning each serotype with its observed prevalence at a given time point or in proportion to the number of individuals in whom it was observed (“ever-carried” model), does not generate results that are comparable with the results from the study. This is because the permutation test generates co-colonisation with a higher frequency than the
sampling and microbiological methods used in this study could generate.

5. If the test would be performed as the authors now propose, the simulation result would be comparable with actual observation, only if the probability of assigning serotypes would depend on whether or not the individual has already been assigned serotype(s). In the ever-carried model, the maximum number of assignment should be effectively restricted to 4. Since such adjustments were not used, the currently employed test statistics has by construction a heavier tail, i.e., towards larger numbers of serotypes ever-carried, than the distribution from which the observed number is a realisation. It is difficult to judge what the impact of this bias is on the difference between the null model and the observation.

6. However, this problem can easily be overcome by adjusting the method. This can be done by allocating the actually observed isolates without replacement to those sampling times at which the children carried pneumococci. For each realisation of such an allocation (i.e. permutation), one would calculate the distribution of different serotypes carried by a child. Taking then average of these distributions over e.g. 100,000 realisations would yield the required reference distribution for the statistical test.

Interpretation of under-dispersion

7. The authors report that the number of serotypes ever carried by the child was less than expected under the null model (“under-dispersed”). There are two possibilities to understand this finding, with several possible underlying explanations. First, the presence of the same serotype in more than one (subsequent) visits can represent a continuing episode of carriage of the same strain. Second, the presence of the same serotype in more than one visits (not necessarily subsequent) can represent a re-acquisition of a circulating serotype. It would be very helpful if the authors could clarify which of the two explanations (or both) they see as the most plausible mechanism. The subsequent discussion on the underlying reasons would then make more sense than now.

8. Specific comments follow: Page 15, in the middle of the page, the reason for bringing up herd immunity here is a bit unclear. Could this be omitted as it does not seem to serve any purpose? In the next sentence, the study by Dagan et al. is discussed. Here it would be worth noting that the study subjects in the Israeli study were somewhat older (toddlers) than the children in the current study, as this is relevant when considering the natural immunity against pneumococci.

9. Still on page 15, when discussing the findings of Goldblatt et al., if this reference is found to be relevant in this context, it should at least be noted that the study subjects in the referred study were all adults. As mentioned in our original review, children less than two years of age mount only low or no antibody response to the polysaccharide capsule (with the exception of response induced by the conjugate vaccines which include an adjuvant). Discussing the role of naturally induced anticapsular antibodies and referring to a study on adults is thus somewhat irrelevant, if it is not made clear that this is of no immediate relevance in the case of children in the age group in the current study.
10. In summary, the chapter starting on page 14 and discussing the reasons for under-dispersion in infants could focus on the phenomena in this age group. Now the chapter elaborates on issues related to vaccine induced immunity in toddlers, naturally induced immunity in adults and previously postulated hypothesis on anatomical and immunological changes taking place later in life.

Minor essential revisions

11. P. 7, line 7: Is 'lgt' meaning 'logit'?
12. The description of the regression models in the methods section is not adequate.
   - On page 7 (line 7), what are the models of ‘odds of carriage’? Is this logistic regression with carriage as the outcome (dependent) variable. If so, please say this.
   - The authors seem to state that logit(prevalence) was then used in all analyses that were based on logistic regression. Is this so?
   - In these analyses, this would mean that
     \[ \text{logit}(\text{prevalence}) = a \cdot \text{logit}(\text{prevalence}) + \text{other explanatory variables} \]
   - Unlike the authors claim, this type of ‘conditioning’ is very unorthodox. If something else was done, it may be ok but was impossible to judge. Please write out clearly what type of regression and conditioning was applied.

12. Page 15: Dagan, not Dangan
13. Half-life of serotypes in infants: 1.8mo (page 17) vs 1.9mo (page 11) in discussion and in results, please clarify and please check the manuscript for other possible inconsistencies

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.