Author's response to reviews

Title: The Epidemiology of Infectious Mononucleosis in Northern Scotland: a Decreasing Incidence and Winter Peak

Authors:

Elizabeth Visser (e.visser@abdn.ac.uk)
Denis Milne (denis.milne@nhs.net)
Ian Collacott (ian.collacott@nhs.net)
David McLernon (d.mclernon@abdn.ac.uk)
Carl Counsell (carl.counsell@abdn.ac.uk)
Mark Vickers (m.a.vickers@abdn.ac.uk)

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Author's response to reviews: see over
To whom it may concern,

Please find our revised manuscript now entitled “The Epidemiology of Infectious Mononucleosis in Northern Scotland: a Decreasing Incidence and Winter Peak”.

Below we set out a point by point discussion of the reviewer’s comments.

Reviewer 1: Klaus Rostgaard

We thank the reviewer for his detailed comments and helpful suggestions.

1. Introduction 4th para/References: Ref 20 (Disanto et al) seems relevant, but is not referenced in the text.
   • This error has been corrected

2. Methods 1st para/References: I fail to see that reference 26 (Taylor GH) is relevant to establish the sensitivity and specificity of the Monospot test. There must be better references, and referenced it must be.

3. Methods 2nd para. You state that 80% of the population were native to Scotland and only 2-4% of the non-natives were born outside EU. Is this also true for the relevant population of primarily teenagers?
   • This data have been updated with the 2011 Scottish census statistics, although specific information on teenagers is not available.

4. Methods 2nd para, last sentence. I suggest rephrasing it something like “An enquiry to the ... ethics committee revealed that no ethics permission was needed for the present study.”
• Text changed to: “An enquiry to the North of Scotland Research ethics committee revealed that no ethics permission was needed for this project”.

5. Methods 3rd para. You are using a t-test to formalize the obvious. I would be happier if you used some non-parametric test instead, since the data are not particularly normally distributed. Anyway this sentence is lacking some detail, it is not clear what is the underlying data points for this exercise.

• The relevant sentence now reads “the difference in ages for males and females was analysed using a Mann-Whitney U non-parametric test”.


• This spelling mistake was corrected.

7. Results 5th para: Given the size of your data material it would have to be a pretty small seasonal signal to go undetected. Therefore the test results in Table 1 borders on the uninformative. I would much rather like to have estimates (with confidence limits) of this seasonality, i.e. (relative) amplitudes and phases. Specifically I would like to know/able to infer how much the fitted constant + sinusoidal intensity varies from top to bottom.. is it 2%, 10% or 40%? Also I would like to know which month the peak occurs in. I think it would be fine to know these things for all the categories by age and sex given in table 1, so that the reader can make comparisons and think up hypotheses.

• Table 1 has been changed to show the peak months, amplitudes (with crude 95% confidence intervals) and the p-values of the total and positive Monospot tests split by age and sex category. The statistical methods and results have been edited accordingly.

8. Results 5th para: I would also like to get an impression of how large a fraction of the variation between month can be ascribed to the sinusoidal which if anything would be associated with vitamin D levels. Some kind of summary measure in a few large strata would suffice. I meant the proportion of variance accounted for by the sinusoidal. I imagine the way to go about it would be to compare deviances for models a (no seasonality), b (only sinusoidal seasonality), c (seasonality by categorical variable month) where \((\text{dev}(a)-\text{dev}(b))/(\text{dev}(a)-\text{dev}(c))\) would be the answer to my question, but if you have a better idea or a better index by all means use that – if you agree with the desirability of providing such information.

• The Edwards’ and Roger’s tests use aggregated data for each month. Upon reading the original text of the statistical analysis section, it suggested that we fitted a model: “Data on negative and positive results were examined to test whether the data fitted a linear or sinusoidal model signifying seasonal variation by using modified Roger’s and Edwards’ tests to clarify the analyses we performed.” We have changed the text
to reflect that we tested for seasonal trends using Edwards’ and Roger’s tests in the statistical analysis section. We then plotted the monthly rates and fitted a sinusoidal curve of best fit through them. The text now read:

“Data on all tests and positive results were tested for seasonal trends using modified Roger’s and Edwards’ tests. The number of tests were plotted by month and a sinusoidal curve of best fit was included [Error! Reference source not found.. Error! Reference source not found.]. These analyses were repeated for different age groups and gender. The peak months, amplitudes (with crude 95% confidence intervals [CI]) and significance levels were calculated from the sinusoidal curves.”

• We clarify that a logistic regression model, as requested above, was not used in this analysis. Although we acknowledge that this would be an alternative method to analyse the data for seasonality as described in:
  Our opinion remains that little extra understanding would be added by using this technique, but we could change our analysis if you argue otherwise.

• Similarly, the uncertainties concerning which vitamin D metabolites are important, the means and distributions of such levels in the at risk population and lag periods are so large, we consider that a mathematical model, which we could provide, would not yield biologically meaningful conclusions.

9. Results 5th para: I also think it would be very useful if you for an online appendix (additional files) created tables (perhaps as spreadsheets) with number of tests, number of positive tests and the background population all distributed by sex, age and month. Such tables would be very useful in future meta-analyses, for power calculations etc. for various copy cats and it would also nicely double as the “materials” table that you haven’t made for this manuscript.

• Additional files added as suggested:

  Table 1: Negative Monospot test results by year, month age and gender
  Table 2: Positive Monospot test results by year, month age and gender
  Table 3: Background Population by year, age and gender from Grampian ISD data [Error! Reference source not found.]
  Table 4: Background Population for the June 2009 Scottish population by age and gender used for standardisation [Error! Reference source not found.]

10. Discussion 2nd para. I think it is fine that you ask your GP colleagues about whether they have changed testing behavior etc. However I think it would strengthen the paragraph (in the eyes of ignorant non-UK residents) if you also discussed the incentive structure here. Who is paying who for
what? Could some of the involved parties have an interest in fewer Monospot tests being performed now? The last sentence (“More objectively…”) should be referenced, if at all possible.

- Text changed to clarify:
  “All IM tests are provided without charge to both GPs and hospital physicians, so there was no financial incentive to change behaviour. More objectively, other haematological blood tests from General Practitioners in Grampian have increased 2.2 fold over this time period.”
  We cannot reference the last sentence; this figure was provided by DM from our laboratory IT system.

11. Discussion 3rd para “….the previous study may have been underpowered…” I think you have the data to decide, rather than speculate about whether this previous study was underpowered.

- This sentence has been removed.

12. Discussion 4th para: Although a small study Tattevin et al (PMID: 16672427) seems relevant too.

- This study has now been added to the discussion

13. Discussion 8th para. The argument about other virus infections in winter as explanations for the IM peak in winter is not entirely clear to me. Actually other virus infections may also have a link to vitamin D and thereby winter. I think that you are right in pointing out all kinds of secular alternative explanations for the seasonal variation. Figure 5 reveals variation by age in the relative seasonal variation in IM incidence. This may suggest that the age groups that are least likely to experience huge population mix-up during a calendar year are the ones that exhibit the nicest, largest (relative) sinusoidal variation in IM intensity. This could be explored and elaborated upon if you made the table suggested above in remark 9.

- We thank the reviewer for suggesting further seasonality analyses.
  Our discussion now reads:
  “For instance, IM may be more common for the same, alternative reasons that infections with other viruses are also more common in the winter months [36]. Splitting the seasonality analyses by age (Table 1) reveals that the effect is strongest at young ages, declines with increasing age and even reverses over the age of 45. Perhaps, therefore, term times may impact on seasonality [5].”

14. Discussion 9th para. Three more seasonality references you should consider: Henke et al. (PMID:4767624), Leard (PMID:4641398) and Rosdahl et al (PMID: 4801052).

- References added as suggested:
Reviewer 2: Hans Joachim J Wagner

We thank the reviewer for the comments.

1. The authors of the study mention in the first paragraph on page 4, that the Monospot test sensitivity and specificity are both nearly 95%. As a reference for this statement they cite a paper from G.H. Taylor about cytomegalovirus infections (Ref 26: Taylor GH: Cytomegalovirus. Am Fam Physician 2003; 67:519-24).
   - The Taylor reference has been replaced with:
     This paper quotes sensitivity and specificity of 93% for the Monospot test.

2. There are numerous older and newer papers indicating that the sensitivity of the Monospot test for the diagnosis of primary EBV infections is less than 95% and reaches a sensitivity of 85%, whereas the specificity is 95-100%.
   - This has been added and referenced in the discussion section.

3. Therefore, the gold standard for the diagnosis of acute and latent EBV infections is the detection of IgM and IgG antibodies against defined EBV-antigens, which were used in this study from 2000-2012. The authors of the study should mention and discuss this aspect in the manuscript. It would be better, if all data were based on serological tests, but these data are not available in this retrospective analysis anymore. At least, the authors should analyze their serological data collected after 2000 with the question if they find the same decrease of IM incidence and changes.
   - Unfortunately the age and sex data for the serological tests were not available for standardisation for this study, thus the suggestion for this analysis could not be performed and we have added this in the discussion section.

We hope the above changes have made our manuscript acceptable for publication.

Yours sincerely,

Dr Elizabeth Visser