PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
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<th>TITLE (PROVISIONAL)</th>
<th>Changing patterns of cytomegalovirus seroprevalence among pregnant women in Norway between 1995 and 2009 examined in the Norwegian Mother and Child Cohort Study and two cohorts from Sør-Trøndelag County: A cross-sectional study</th>
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<tr>
<td>AUTHORS</td>
<td>Odland, Maria; Strand, Kristin; Nordbø, Svein; Forsmo, Siri; Austgulen, Rigmor; Iversen, Ann-Charlotte</td>
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VERSION 1 - REVIEW

| REVIEWER            | Michael J. Cannon  
|                     | Research Epidemiologist  
|                     | National Center on Birth Defects and Developmental Disabilities  
|                     | Centers for Disease Control and Prevention  
|                     | USA |
| REVIEW RETURNED     | 14-May-2013 |

REPORTING & ETHICS

There is a mention of obtaining participant consent but I did not see a mention of having obtained ethical approval from a human subjects research board.

GENERAL COMMENTS

This interesting article addresses the prevalence of and risk factors for CMV seropositivity in Norway, a population that is understudied. Also, the article is one of only a few that has evaluated temporal changes in CMV seroprevalence. I have several relatively minor comments.

Comments

1. Because changes in seroprevalence were an important focus of the article, additional citation and discussion of studies of trends in seroprevalences are warranted, for example, Marshall, Am J Perinatol (2005); Bate, Clin Infect Dis (2010); Lubeck, Med Microbiol Immunol (2010); Inde, J Obstet Gynecol Res (2010).

2. I am not sure that it is appropriate to refer to the study as a national population-based design in the Strengths and Limitations and in the Discussion. Only Study Group 1 is national, and it represents only the 38% who self-selected to participate in MoBa, although the authors do acknowledge this as a limitation. Perhaps a more questionable decision in this regard was to exclude the 43 women from SG 1 whose first language was not Norwegian. Although they represent only 4.3% of the overall study population, it is not clear why they should be excluded. If the authors wish for their sample to represent Norway as a whole, then non-native speakers ought to be included in the analyses.
3. In the Methods the authors describe the multivariate analyses, including logistic regression models with multiple variables as confounders. However, I could not find a thorough description of the multivariate results. Instead, the Results only alluded to these analyses (Lines 220-228) and did not explain how final models were chosen (e.g., adjusted for education in line 221 but did not seem to adjust for other confounders). The authors need to put the multivariate results into a Table (either add to an existing one or create a new one) and justify why they used the model variables they used.

4. Lines 275-278: It seems unlikely that this change in ethnic composition could contribute much to the time trend. My back of the envelope calculation would suggest that at most, if the non-ethnic CMV seroprevalence were 100%, the 3% increase in non-ethnic Norwegians would only lead to just over a 1% increase in CMV seroprevalence. So, it would be important to point out that changes in ethnicities are unlikely to explain much of the seroprevalence trend. It seems possible that age changes could be partially responsible for the increase as well, given the 79% CMV seroprevalence in the youngest group. Perhaps the authors could explore whether the proportion of pregnancies occurring in the <25 group has increased during the time of the study.

5. Line 301: Do you have data from the 1970s to confirm the increase? Are they addressed in reference 32, or does that reference just talk about current percentages?

6. Tables 1 & 2: Clarify that High educational level refers to university (if that is the correct interpretation). Also, show the income values in parentheses for the ease of the readers.

REVIEWER
Pat Tookey
Senior Lecturer
UCL Institute of Child Health
UK

I have no conflicts of interest.

REVIEW RETURNED 19-Jun-2013

THE STUDY
Most of the issues on the score sheet were reasonably dealt with, but I have a few comments and questions. This is an interesting paper exploring differences in CMV seroprevalence in the Norwegian pregnant population, between groups and over time. The authors conclude that some of the differences observed could be attributable to changes over time in the pregnant population’s own breastfeeding history and attendance in day care – however, these issues are only raised in the closing section of the discussion, and are not flagged up in the key messages or in the introduction. Although this contemporary analysis of Norwegian data is valuable, this is not a new observation and evidence for the role of breastfeeding and day care has been
presented by other groups. However, only two specific references (30 Hamprecht, and 31 Pass) are included here, and I wonder if the authors could bring these issues more to the fore earlier in the paper, and build on the strength of their data being contemporary (many of the earlier studies are quite old)? Further useful references and discussion can be found in various review and research papers, including Joseph et al. Cytomegalovirus as an occupational risk in daycare educators. Paediatr Child Health 2006;11(7):401-407 Peckham et al. Early Acquisition of CMV infection. Arch Dis Child 1987;62:780-785 might also be useful with respect to early acquisition of CMV in breastfeeding populations.

Introduction
1. 76-77 - Ref 7 provides US estimate of incidence of primary infection in pregnancy in different population groups (Mexican, non-Hispanic white, non-Hispanic black). Any northern European/Scandinavian estimates?

Methods
1. lines 165-167 (and 206-211) Exclusion of women with equivocal results. Is it likely that equivocal results could represent early stages of primary infection? Maybe needs comment in limitations if so?
2. line 181 - women with missing data were excluded from the analysis. Might this have introduced further bias on top of the participation issues, and could this also be discussed in limitations?
3. Statistical methods and analysis – lines 179-181 mentions multivariate models, but from Table 2 it looks as if each variable was adjusted for age only. Is this correct? In the results there is discussion of geography being adjusted for education, but this is not presented in the table although it is of interest... was this analysis also age-adjusted? Adjustment for smoking is also mentioned in both methods and results, though not presented in the table. But maternal smoking, infant birth weight and prematurity are all related to variables which are already included in the analysis (SES, income, age) and not independently with maternal CMV seroprevalence itself, so although they are of interest with respect to representativeness of MoBa, I’m not sure they are needed in the multivariable analyses. Could the authors give a bit more information about their analyses, and clarify how they developed their models and which variables were included?

Minor typos/language issues
Line 100 economy = socioeconomic status or income?
Line 121 Conclusively = Therefore
Line 200 less premature babies = fewer premature babies
Line 273 Conclusively = It is therefore likely that
Line 276 Missing words? We cannot exclude the possibility that the
Line 308 Low education = fewer years of education

Tables: Could Tables 1 and 2 be combined, much information overlaps? Age group categories are different in the 3 tables, and not always exclusive (eg 25-29/25-30; 30-34/31-35; 30-34/>35) Table 1: Clarify this is proportions not numbers – but they don’t agree with data provided in Table 2 which is a bit confusing. Table 2: Specify number with available data for each variable (848-921, but reader needs to calculate).

How many women were included in the adjusted analyses?

RESULTS & CONCLUSIONS
The data are consistent with the authors’ contention that maternal breast feeding history and experience of day care may have an impact on CMV seroprevalence in pregnancy, and changes over time in the Norwegian population.
But the argument would be stronger and clearer if other supporting data (more national data on use of day care, or rates of breastfeeding, similar findings from elsewhere) were provided. The geographical variation is interesting – is it mostly explained by age, education and income variables? Could it also be that breastfeeding history and/or use of day care varies by geography?

There is a reasonable discussion of some of the limitations of the study.

Do these observations have any implications for rates of congenital infection? Is there any published data on congenital CMV rates in Norway either recently or over time?

VERSION 1 – AUTHOR RESPONSE

REPLY TO REVIEWER #1; Michael J. Cannon:

Ethical approval. Please find the description of the ethical approval by the Regional Committee for Medical Research Ethics in South Eastern Norway for the use of Study Group 1 (from the Norwegian Mother and Child Cohort Study) in the Ethics approval section of our manuscript in page 16, line 350-354. Since Study Group 2 and 3 are based on anonymous quality assessment and ethical approval for research purpose is not requested according to the Norwegian Health Research Act, a sentence describing this has been added to the Ethics approval section (page 15, line 354-357).

Comments:

1. Reviewer #1 requests citation and discussion of relevant studies and we would like to thank the reviewer for his suggestions and have added the suggested articles to the manuscript. The reference list numbering has been changed accordingly.


2. Reviewer #1 questions whether it is correct to refer to the study as a national population-based design in the Strengths and Limitations part of the Discussion. We acknowledge the Reviewers comment, and have now modified our text to show that only Study Group 1 is a national population-based study group, and “of Study Group 1” has been added to the sentence “The strength of this study is the national population-based design of Study Group 1 and the opportunity to assess regional differences in CMV-IgG seroprevalence” on page 12, line 263-264. Reviewer #1 also questions our decision to exclude non-native speakers and we appreciate this important comment. The non-native speakers were excluded because they might represent a group with different CMV seroepidemiology.1-2 The number of immigrants in Norway is still rather low and quite unevenly spread across the country, and including them would potentially make the regional assessment of differences in CMV-IgG seroprevalence among Norwegian women more unreliable. Before exclusion of non-native speakers, the overall CMV-IgG seroprevalence in Study Group 1 was 60.5% (95% CI 57.4 to 63.6) (n=965), and CMV-IgM seroprevalence was 1.2% (95% 0.6 to 2.1) (n=990). The exclusion of non-native speakers did therefore not change the overall result, but due to the reasons given above, we hope that the reviewer agrees with our decision to exclude this small group from the analyses.
3. Reviewer #1 points out that the Methods section describes multivariate analyses without a thorough explanation of these results later in the manuscript and we sincerely apologize for this mistake. We originally have adjusted the data for several different confounders that could potentially affect the CMV-IgG seroprevalence, however since none of these changed the results significantly, only what are considered the most important confounders were presented and discussed in the submitted manuscript. We have therefore changed line 187-189 in the Statistics section on page 8 according to this, and adjust for only maternal age and education (Table 2), since they are two important factors that may influence CMV-IgG seroprevalence. We now only describe adjusting for the confounders that are presented in Table 2. We hope the Statistics section is now properly in line with what we describe further in the Results section.

4. Reviewer #1 has some suggestions regarding our discussion of ethnic composition. We would like to thank Reviewer #1 for this interesting observations and propositions on ethnicity and age of pregnant women in the Sør-Trøndelag Study Group 2 and 3, and highly appreciate that the suggestions strengthens our findings. We have included the following paragraph in the Discussion section on page 13 in line 287-295 “However, a 3% increase in non-ethnic Norwegians would lead to only about a 1% increase in CMV-IgG seroprevalence, leaving a change in the ethnic composition an unlikely explanation for the increasing trend in CMV-IgG seroprevalence over time. The youngest women <25 years had the highest CMV-IgG seroprevalence and the increasing trend in CMV-IgG seroprevalence could possibly be due to an increased proportion of young pregnant women in the population. However, in line with an increasing mean age of delivering women in Norway (1995: 28.3 years, 2009: 29.7 years), the proportion of delivering women <25 years of age in Sør-Trøndelag County was 24.6% in 1995 and only 16.7% in 2009”.

5. Reviewer #1 requests data from the 1970s confirming the increase in the use of day-care center and breastfeeding in Norway. We have included such references in the manuscript, but unfortunately, the most relevant references number 41 and 43 are in Norwegian, but both include graphic presentations of this increasing use of day-care centers after the 1970s. Reference 42 and 45 describes the increase of breastfeeding during the last decade, whilst references 43 and 45 describe the current use of day-care center and breastfeeding.

6. Tables 1 & 2: It has been clarified in table 1 and table 2 that high educational level refers to university education, and the income values are shown in parentheses as Reviewer #1 correctly requested.

REPLY TO REVIEWER #2; Pat Tookey:

General comments:

Reviewer #2 suggests focusing more on breastfeeding and use of day-care centre earlier in the Introduction and in the Key messages. We would like to thank the Reviewer for this valuable advice. Line 93-96 page 5 in the Introduction have been included to bring forward the possible impact breastfeeding and use of day-care centre may have on CMV-IgG seroprevalence in developed countries; “CMV is known to be transmitted through breast milk, and infected infants shed virus in the urine years after inoculation. Thus increased use of breastfeeding and day-care centre are suggested reasons for higher CMV-IgG seroprevalence in some developed countries, such as Sweden.” The subject is now also included in the third Key message point (page 3, Line 58-59); “CMV-IgG seroprevalence of pregnant Norwegian women seems to have increased during the last years, and possible explanations may be the increasing use of breastfeeding and day-care center in Norway since the 1970s”. However, we have included moderate text additions, because investigating the association between use of breastfeeding/day-care centre and CMV infection was not an original aim.
of study, but instead become an interesting interpretation of our findings.

Reviewer #2 would also like us to build more on our data being contemporary, a good advice we have tried to adhere in the Discussion on page 14, line 322-328; “A relation between high breastfeeding rates and the use of day-care centre in developed countries has previously been suggested as a reason for high CMV-IgG seroprevalence rates, but updated Norwegian studies supporting this have been missing. This study provides contemporary data on an increase in CMV-IgG seroprevalence in Norwegian pregnant women supporting this hypothesis.”

The reviewer further suggested including two more references. We would like to thank for the information and the additional references (reference number 26 and 27) are now included in the manuscript in page 5, line 93-96.

Introduction
1. Reviewer #2 was missing a Scandinavian estimate on primary infections among CMV-IgG seronegative women in pregnancy. An estimate from the largest study by Ahlfors et al (reference 8) on CMV-IgG seroprevalence in Sweden is now included on page 4, line 77-78 in addition to the US estimates.

Methods
1. Reviewer #2 suggests that women with equivocal results could represent early stages of primary infection. However, the CMV-test used detects IgG antibodies later than the IgM antibodies during a primary infection, and no CMV-IgM antibodies were detected in sera with equivocal CMV-IgG results. Hence, we consider that it is not likely that these results could represent early stages of primary infection and hope the Reviewer agrees with us.

2. Women with missing data were excluded. Reviewer #2 wonders if this could have introduced further bias upon the participation issues. This issue is now discussed in the limitation section of the Discussion on page 12, line 279-282; “Missing data among some women in Study Group 1 could potentially bias the results of the multivariate logistic regression analyses. These women, however, have a higher CMV-IgG seroprevalence and are most likely lesser educated, hence the associations presented are underestimates rather than overestimates.”

3. Statistical methods and analysis. Reviewer #2 requested more information about the analyses and clarification of what variables were included, and again we sincerely apologize for the confusion made in the manuscript. Please see the response to Reviewer #1 point 3 and the according change in the Methods section page 8, line 187-189. Maternal smoking, birth weight and prematurity are, as the Reviewer #2 also states, not independently related to CMV-IgG seroprevalence. Therefore they are not included in multivariable analysis. Instead they are used to describe Study Group 1, and by so enabling comparison to the general pregnant population of Norway.

Minor typos/language issues
The minor typos/language issues Rewiever #2 pointed out have been corrected;

Line 106 Economy = Income
Line 128 Conclusively = Therefore
Line 205 Less premature babies = Fewer premature babies
Line 277 Conclusively = It is therefore likely that
Line 285 Missing words = We cannot exclude the possibility that the
Line 331 Low education = Fewer years of education
Tables
a. Reviewer #2 correctly notices some flaws in the tables that have now been corrected. In Table 1 we have included numbers in addition to proportions and we have changed the maternal age groups making it similar to Table 2 and 3, and we would very much like to keep Table 1 as a separate table. We hope the tables are now clearer to the reader. We have also specified the number of women with missing values for each variable instead of the number of women available for analysis for each variable, in order to make the table as easy to read as possible.

Discussion
a. Reviewer #2 is requesting more supporting data on breastfeeding and use of day-care centre. Please see the response to Reviewer #1 point 5 where this question has been addressed by including more references.
b. Reviewer #2 is asking for whether geographical variations could be a result of differences in breastfeeding rates and use of day-care centres. This has been considered a contributing factor, however, the regional differences are too small to explain the results. This is now mentioned in the Discussion on page 14, line 327-328; “There are also regional differences in breastfeeding and the use of day-care centre in Norway, but these are too small to explain the differences in CMV-IgG seroprevalence7”, and reference 46 has been included showing the variation between Norwegian counties in use of day-care centre. Reference 44 shows both the increase in day-care centre after the 1970s and the use of day-care centre in the different counties in Norway (only numbers and not proportions). We were unfortunately not able to achieve information on breastfeeding rates by different counties, but this is not expected to vary much.

Reviewer #2 wonders if the changes in CMV-IgG seroprevalence could have any impact on the rate of congenital CMV infection in Norway and we appreciate the importance of addressing this question. Reviewer #2 also wonders if there are any published data on congenital CMV from Norway. When investigating CMV-IgG seroprevalence in Sør-Trøndelag County in 1995, congenital infection was found in 0.3% of the infants (unpublished material), but updated Norwegian data supporting this is unfortunately not available. However, a large study from Sweden done in 1982 isolated CMV from 0.5% of the newborn babies.8 It has been suggested that a higher CMV-IgG seroprevalence means more circulating virus in the surroundings, potentially causing more congenital infections. If this is the case in Norway, we can expect more congenital infections in the future, but the data are too limited, and since this is considered beyond the scope of this study, we have not added this otherwise important topic to the Discussion.

We highly appreciate the opportunity to submit this revised manuscript for consideration of publication in BMJ Open.

On behalf of the authors,
Sincerely,

Maria Lisa Odland, medical student, PhD-program.

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VERSION 2 – REVIEW

| REVIEWER | Michael J. Cannon  
|          | Research Epidemiologist  
|          | Centers for Disease Control and Prevention  
|          | USA  
| REVIEW RETURNED | 08-Aug-2013

THE STUDY

The authors’ response to my comments was helpful and appropriate. I have one remaining question. The adjusted results in Table 2 still don’t make sense to me. In one column the authors say they are adjusted for age while in the other column they are adjusted for education. But it seems to me that in each column they are adjusted for all the variables simultaneously except for age (in the first adjusted column) and education (in the second adjusted column). Otherwise how do they come up with results for parity or income or place of residence? So, assuming that is what they did, they should accurately state that in the footnotes to the table, rather than stating that they only adjusted for age or education.

VERSION 2 – AUTHOR RESPONSE

Comments:
1. Reviewer #1 is still wondering about the adjusted results in Table 2. We have now included a footnote in the table, see line 516-517. We have also included the following text line 182-188;

All seroprevalence data were assessed as proportions (percent) with confidence intervals (CI) of 95%. Univariate differences in the proportions between groups were tested by Chi square, and logistic regression was used to calculate crude and adjusted odds ratios (OR) of CMV seroprevalence by selected predictors in bivariate and trivariate regression models. The predictors were place of residence, age group, parity, educational level, maternal income and family income. Maternal age and education are both factors known to influence CMV seroprevalence and were adjusted for by logistic regression.
Changing patterns of cytomegalovirus seroprevalence among pregnant women in Norway between 1995 and 2009 examined in the Norwegian Mother and Child Cohort Study and two cohorts from Sør-Trøndelag County: a cross-sectional study

Maria Lisa Odland, Kristin M Strand, Svein Arne Nordbø, Siri Forsmo, Rigmor Austgulen and Ann-Charlotte Iversen

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