Dear Ms. Emma Veitch,

Thank-you for the comments regarding our manuscript. We were impressed by the caliber of the reviewers and are pleased to respond to their recommendations.

Comments by Reviewer # 1, Dr. J. Thomas Grayston

1. We have re-written the results and conclusions sections of the abstract as follows:

Results: Eighteen relevant studies were identified. In nine CV studies with control subjects, the prevalence of circulating C. pneumoniae DNA was 252 of 1763 (14.3 %) CV patients and 74 of 874 (8.5 %) controls, for a pooled odds ratio of 2.03 (95 % CI: 1.34, 3.08, P 0.001). Prevalence was not adjusted for CV risk factors. Current smoking status, season, and age were associated with C. pneumoniae DNA detection. High prevalence (> 40 %) was found in patients with cardiac, vascular, chronic respiratory, or renal disease, and in blood donors. Substantial differences between studies were identified in methods of sampling, extraction, and PCR targets.

Conclusions: C. pneumoniae DNA detection was associated with CV disease in unadjusted case-control studies. However, adjustment for potentially confounding measures such as smoking or season, and standardization of laboratory methods, are needed to confirm this association.

2. We emphasized season and smoking, since these are strongly associated with C. pneumoniae DNA detection, and with cardiovascular disease, and therefore are potentially important confounders of an association between C. pneumoniae DNA detection and cardiovascular disease. We agree that age and gender may be potentially important, and have added these in the manuscript as follows:

p. 11: Further studies with concurrent controls, matched or adjusted for age, gender, smoking and season, are required to conclude whether C. pneumoniae DNA detection is associated with
cardiovascular disease.

p. 13: In summary, these data suggest that C. pneumoniae DNA detection in PBMC should be performed, as a minimum, during winter and spring months, and controlled for smoking status. Any comparisons with controls should also be controlled for age and gender, although the relationship between these factors and C. pneumoniae DNA prevalence remains unclear.

3. The reviewer cautions about the inclusion of abstracts. However, this is traditional in meta-analysis, since negative results tend to take longer to get published or may never get published at all. There were 4 abstracts cited in the paper, two of which are now published; Sessa et al and Freidank et al. Our a priori protocol was to include identified abstracts, although not all data was included in the meta-regressions if some details were missing. We feel it remains important to include the remaining abstracts in this review.

4. We have followed the reviewer's recommendation to remove the unpublished study (reference 26) from the table and compilations. We have adjusted all analyses correspondingly, including Table 1 and Figure 1 and 2. As the reviewer suggests, we have included a brief discussion of the unpublished study in the discussion:

p. 11: However, lack of adjustment of potentially confounding variables such as smoking, season, age and gender may negate these results. Indeed, in a recent study of 310 coronary angiography patients and 102 concurrently-recruited family practice controls, adjusting for smoking and season, we found that C. pneumoniae DNA prevalence was less common among heart disease patients than in controls (Smieja et al, unpublished). Addition of this unpublished study would result in a non-significant association between C. pneumoniae detection and cardiovascular disease (pooled OR = 1.6, 95% CI: 0.7, 3.5, P = 0.22).

5. The shape of the funnel plot (Figure 2) is highly suggestive either of publication bias, or of bias in selection of cases or controls. This plot is important, in that an epidemiologist will have much less confidence in these results than if the studies were symmetrically distributed about the actual pooled odds ratio. Publication bias remains possible, since we did not contact authors who were not already well-known in the field, and our search for unpublished data was relatively cursory. We have not changed the manuscript in this regard.

6. We have removed the duplicate reference and renumbered all references according to standard citation methods.

Comments by Reviewer #2, Dr. Rosa Sessa

1. Reviewer #1 (Dr. Grayston) recommended that the single outlier study (manuscript in preparation) be removed from the main analysis. We have removed the outlier study as an unpublished study from both the results and methods, and included it in the discussion. The study will be submitted this month for publication, and much more detail will be found there. Please see point number 4 under reviewer one.

2. We have altered the discussion to emphasize the importance of adjusting for confounding variables, and only a limited discussion of the outlier study (see above).

p. 11: Using meta-analysis, we demonstrated that circulating DNA prevalence was higher among cardiovascular patients than among controls. However, lack of adjustment of potentially confounding variables such as smoking, season, age and gender may negate these results. Indeed, in a recent
study of 310 coronary angiography patients and 102 concurrently-recruited family practice controls, adjusting for smoking and season, we found that C. pneumoniae DNA prevalence was less common among heart disease patients than in controls (Smieja et al, unpublished). Addition of this unpublished study would result in a non-significant association between C. pneumoniae detection and cardiovascular disease (pooled OR = 1.6, 95% CI: 0.7, 3.5, P = 0.22). Further studies with concurrent controls, matched or adjusted for age, gender, smoking and season, are required to conclude whether C. pneumoniae DNA detection is associated with cardiovascular disease.

3. We have altered our conclusions to emphasize solutions to the summarized problems, as follows (p. 17):

The detection of C. pneumoniae DNA in PBMC was associated with CV disease in unadjusted case-control studies, as summarized in this review, and is a promising test for further molecular epidemiologic studies. To verify this disease association, future studies will need to match or adjust for potential confounding by smoking, season, age, and gender. To ascertain the role of C. pneumoniae in cardiovascular disease and other chronic diseases, epidemiologists and other researchers need a sensitive, specific, and reproducible test that correlates with the endovascular presence of C. pneumoniae. To improve the sensitivity of PBMC testing, we recommend using the nested MOMP PCR and sampling large blood volumes. Studies are needed to optimize blood volumes and extraction methods, and to further validate circulating C. pneumoniae DNA detection as a surrogate marker for its presence in atheroma.

4. We have corrected the citations as suggested, and thank Dr. Sessa for her careful scrutiny of the manuscript. We made the following changes:

   a) The two references cites as abstracts have been changed to a citation of the published abstract (Sessa and Freidank).
   b) Unpublished data is cited in parentheses in the manuscript.
   c) Tables include reference numbers. We have not included references for the methods, as this is not a primary focus of this paper.
   d) Corrected the percentages for the data from Rassu et al:

   p. 7: Among blood donors, prevalence was 16.7 % in Australia (34), 46.2 % in Italy (35), and 8.9 % in the United States (36).

Comments by Reviewer #3, Dr. M. Leinonen

There are no specific comments requiring a change in the manuscript. We agree that a systematic review of chlamydial serology would be worthwhile, however this is well beyond the scope of this manuscript.