Reviewer's report

Title: Ureaplasma parvum infection alters filamin A dynamics in host cells

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Reviewer: Christine L Knox

Reviewer’s report:

This report details comparative proteomic profiling of bladder tissues from F344 rats infected with U. parvum and uninoculated control rats. These experiments clearly show differences in expression in a range of proteins, including filamen A, in association with ureaplasma colonisation. Of great interest, differences in filamen A expression were also demonstrated in a human benign prostate epithelial cell line after incubation with ureaplasmas. These preliminary findings will further help researchers to investigate the histological presentations associated with ureaplasma infection/colonisation, which range from asymptomatic infection to severe inflammation.

Major compulsory revisions

1. abbreviation for Ureaplasma parvum is U. parvum. In the article the abbreviations UP or U. parvum have been used – these should all be standardised to U. parvum.

Clarification of:

2. preparation of tissues for proteomic studies. Were excess tissues snap frozen at the time of necropsy after experiments reported by in BMC Infectious Diseases Reyes et al. 2009 and then processed later for these experiments? Or was protein extracted from tissues at the time of necropsy and then frozen?

3. the numbers of tissues that were included for proteomic analysis for each experimental group: -Control, Negative, UTI and Struvite. Three iTRAQ experiments were performed for each group- does this mean that one sample ('a representative sample') from each group was tested in each experiment – so that a total of 3 tissues was tested for each group?

4. Minimizing variability between protein load between groups only tissues with a similar culture CFU (2.4- 2.7) at time of necropsy were chosen – does this mean that the tissues were cultured and those found to have 2.4- 2.7 CFU/g were selected for the UTI and Struvite group? If so then was a matched control selected from the Negative group, i.e. a Negative animal that was injected with the same dose of ureaplasmas (doses ranging from 101 - 109)?

5. Additional table 1: This includes information relating to the 28 proteins that were different for the Negative group and the colonised animals (UTI and Struvite). However the means (what are these, are these means of the protein ratios??) for the control and the negative group were not included in this table, only those for the UTI and struvite groups. The colour coding in this table – does
red refer to means that were higher for the struvite group compared to UTI? If so then the first entry ‘ApoH Apoliprotein H’ – should be green

6. Table 1 – What is the effect? Are these the differences between proteins in the negative group versus colonised groups or between the UTI versus struvite group? – the latter correspond to the results reported under ‘effect’ in Table 1

7. Figure 1 C – clusters should be identified within the figure.

8. Figure 2
   o In the text p 14 – Intracellular distribution of filamin A in BPH-1 cells is Figure 2 A- H not Figure 3 as recorded in text.
   o Was ureaplasma IFA performed on the cells in panels D, E, F? If so could an additional panel be included to demonstrate the location of the ureaplasmas?
   o In 2H – the intensity of the filamin A staining appears decreased compared to G – this is also consistent with the ELISA results.
   o Prominent staining of filamin A within the cytoplasm ‘(Figure 2E and 2F)’ – also to be changed.
   o Panels G and H – these were incubated for 72 hours with supernatants obtained from BHP-1 cells incubated for only 24 hours (this should be included in the figure legend) with ureaplasmas or 10 B media. Why was a 24 hour supernatant used rather than a 72 hour supernatant? Could this account for staining differences?

9. Last paragraph p 14. To further characterize the condition of filamin A in infected BPH-1 cells, cell lysates were evaluated by- changes to ‘ELISA (Figure 3) and western blot (Figure 4)’. Please correct figures numbers throughout the next 2 paragraphs.

10. Change Figure 6 – to Figure 5. Was the staining of phosphorylated filamin A performed on cells incubated with supernatant only? Can an image be shown?

The inclusion of some of these changes may lead to changes in the discussion.

Minor Essential Revisions

11. The paper would benefit from proof reading and editing, with particular attention to the consistent use of units of measurement: ml, 0 C, g, CFU ?per ml or per g -

12. page 12 the first sentence of the results section could be rephrased to help the reader interpret the meaning.

Discretionary revisions

13. Background – p3 paragraph 2 would be best written in the past tense.

14. Are any commercial antibodies available for the rat proteins identified in the proteomic studies? It could be interesting to further explore the filamen A distribution in tissues with different grades of histological inflammation. It could be useful to comment on possible studies in animal model studies.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests