Reviewer’s report

**Title:** Human isolates of Bartonella tamiae induce pathology in experimentally inoculated immunocompetent mice

**Version:** 2  **Date:** 19 April 2010

**Reviewer:** Michael Minnick

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The manuscript entitled “Human isolates of Bartonella tamiae induce pathology in experimentally inoculated mice” by Colton et al. describes the pathology induced by a newly-described, pathogenic Bartonella species in the context of a murine model. While the data are interesting and the observations promising, the study could be improved in several areas:

**Major Compulsory Revisions—**

1) Three strains of B. tamiae were employed and used to inoculate 4 mice per strain. Subsequently, one mouse was euthanized and analyzed at four post-inoculation time points (3, 4, 5 and 6 weeks). As a result, n = 1 for each time point per strain. This n value is clearly not adequate for the study. There is also some reference to n values of 4, but this is technically not correct (see page 16, line 8; page 17, line 15).

2) It is not clear whether viable bacteria are even necessary for the observed pathology, since PCR was used to detect B. tamiae DNA. A non-viable control (e.g., irradiated bacteria) would address this concern and improve the study.

3) Does B. tamiae grow in mice? Use of PCR to test for DNA is fine, but without a dead bacteria control, the authors’ positive PCR results may have arisen from residual DNA from the original inoculation. An attempt to culture the bacterium from the test animals would be additional proof for the utility of the murine model.

4) No indication or safeguards are mentioned regarding the possibility that another co-inoculated pathogen was responsible for the observed lesions. This is especially important since B. tamiae was never cultured from challenged mice.

5) It seems very odd that the most “virulent” strain (Th239) is not detectable in tissues by PCR at any time point in the study. Without the controls suggested above, it is difficult to explain this observation.

6) The authors are cognizant of age-related bias in animal studies. Ironically, they state “aged immunocompetent” on page 19, line 3; but older animals are generally more prone to infection and utilization of mice aged 15-18 months (quite old) biases the study. The authors should justify why this age group was chosen for their study.
7) Figures- The authors arrow a myocyte with a pyknotic nucleus (Fig. 1) and a necrotic hepatocyte (Fig. 2). Were these cell types common or rare in the sections? How many sections were prepared? It is difficult to extrapolate pathology from one cell of these types in one section with an n = 1.

Minor essential revisions-

Table 1 should be deleted and the 4 primers moved to the Methods text.

Page 3, line 13- “…study the pathogenesis of these…” The current model is only useful for pathology. Until the authors show that B. tamiae grows in the mouse, it can’t really be used as a model for pathogenesis.

Page 3, lines 16-17- Proteobacteria is a phylum (not a family). Bartonellaceae is the family.

Page 6, line 1- Please provide in vitro passage number (away from humans) for the strains.

Page 6, line 7- mice were inoculated subcutaneously. The authors should briefly explain this choice for route of challenge. Also- did they make attempts to simulate hematophagous insect bites or was the injection into the fascia (truly subcutaneous)?

Page 10, line 15- “did not appear painful”- the meaning of this is unclear.

Page 11, line 13 and Page 14, line 10- The authors shouldn’t use the term “virulent” without doing ID50 or LD50 studies in the murine model. The term pathogenic should be used in its place.

Page 13, line 6- A figure showing the skin lesions could be useful, as this might be a possible route of challenge in humans. The image may also be helpful for superficial diagnostics in the clinical setting. If a figure is not provided, add “data not shown”.

Table 3- Define the acronym “NAF” in the table legend.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'