Author’s response to reviews

Title: Brucellosis caused by the wood rat pathogen Brucella neotomae: two case reports

Authors:
Juan Villalobos-Vindas (juanvillaloboscr@gmail.com)
Ernesto Amuy (eamuy@yahoo.com)
Elías Barquero-Calvo (elias.barquero.calvo@una.cr)
Norman Rojas (norman.rojas@ucr.ac.cr)
Carlos Chacon-Díaz (CARLOS.CHACONDIAZ@ucr.ac.cr)
Esteban Chaves-Olarte (esteban.chaves@ucr.ac.cr)
Caterina Guzmán-Verri (caterina.guzman.verri@una.cr)
Edgardo Moreno (edgardo.moreno.robles@una.cr; emoreno@racsaco.cr)

Version: 1 Date: 28 Sep 2017

Author’s response to reviews:

Dr. Rajinder Bajwa
Editor
Journal of Medical Case Reports

Dear Dr. Bajwa,

I am submitting for your consideration the revised version of the article entitled “Brucellosis caused by the wood rat pathogen Brucella neotomae: two case reports”. We have answered all the reviewers’ queries in detail and indicted in the response letter the specific sections that have been modified in the manuscript, accordingly. Following the reviewers’ suggestions we have thoroughly revised the all sections of the manuscript.

We are thankful to both reviewers for their thoughtful comments and suggestions on the manuscript, which we thoroughly considered. We have answered all queries in the same order as they were raised.

Sincerely yours
Edgardo Moreno

Response to reviewers:

Reviewer #1:

Comments:

- In case 1, It is not clear when the initial blood cultures were drawn and when the second set were drawn. (lines 79-83)

This has been explained. Page 4, lines 82-85

- Line 76: suggest revision to say echo did not reveal any vegetation or other sequelae of IE rather than ruled out

Change has been done. Page 4, lines 77-78.

- Line 89: Is the drug regimen correct? the drugs and respective durations seem to have been juxtaposed

The reviewer’s comment is correct. The sentence has been corrected. We apologize for the mistake. Page 4, line 94; page 5, line 95

- Case 2: no mention of any CSF studies are made.

In the previous manuscript describing the genomic characteristics of the isolate we made an unintentional mistake. The clinical report performed at the hospital, described case number 2 as a presumptive neurobrucellosis; however, we have verified that in case number 2 no CSF culture was performed whatsoever. Therefore, the clinical report described in the present manuscript, is correct. Although, this mistake does not change the interpretation of our results, we will submit a note to the J. Emerg. Dis. clarifying this. We sincerely apologize for this error in previous manuscript.

- the WHO guidance re treatment for Neurobrucellosis usually recommends 3 drug regimen. can the authors comment on the treatment used in their cases.

Although different treatments have been recommended for neurobrucellosis, it has been demonstrated that treatments with three drugs do not give any additional advantage over the conventional treatment with two antibiotics. See: Ariza PlosMed, 2007; 4: e317

- Line 133: minor changes in blood counts are common, especially low WBC or platelet count along with coagulation deficiencies. authors may need to update their references to more recent date publications (reference 9).
Minor blood cell and platelets changes are observed in just one third of the brucellosis cases. Coagulopathies are rare. The hematological and pathophysiological conditions in a large number of brucellosis cases have been described just by some authors. Some of the most reliable data were described by Spink, Ruiz-Castañeda and Pons, all classical references in which large number of brucellosis cases were studied. All of them agree with this profile. Recent references (e.g. Lancet Infectious Diseases 7: 775) with less number of cases support the observations of Spink. Following this, and for the sake of strictness, we have kept the reference of Spink and added newer references, complementing the classical Spink reference. Page 6, line 137-138.

- Line 130: authors state the bacterium was shown to have crossed the BBB but do not give any proof of CSF analysis in case 2.

We agree with the reviewer’s criticism. The sentence has been changed accordingly to present only one case of neurobrucellosis. See Page 6 lines 140-142.

- Line 228: References are out of sequence (7 before 6)

It has been corrected. Page 11, lines 243-247.

- Need to acknowledge prior publication of these 2 cases (Suarez-Esquivel M et al, PMID: 28518028)

Although we have clearly referred our previous work in various paragraphs, we have made obvious our contribution in Emerg. Infect. Dis., in the first sentence of the Discussion. Page 6, lines 128-130.

Reviewer #2:

Do you have any ethical concerns? Please consider if local Institutional Review Board approval or ethical approval was obtained (if appropriate) and if the patient (or their parent or guardian in the case of children under 18) gave written, informed consent to publish this case and any accompanying images. A statement to this effect should appear in the manuscript.

Comments:

This is the second report of the two patients (and they differ in some regards)

In the previous manuscript, describing the genomic characteristics of the isolate, we made an involuntary mistake. The clinical report performed at the hospital, described case number 2 as a presumptive neurobrucellosis; however, we have verified that in case number 2 no CSF culture was performed whatsoever. Therefore, the clinical report described in the present manuscript, is correct. Although, this mistake does not change the interpretation of our results, we will submit a note to the J. Emerg. Dis. clarifying this. We sincerely apologize for this error committed in our previous manuscript.
Comments: It is not clear why there was a delay of 5 years between isolation and identification of the organism. (see comments below for other issues)

Following the reviewer’s comment, we have made clear the reason for this delay. Page 6, line 130-136.

1) The two patients were already reported in your paper in Emerg Infect Dis (June 2017) and there appears to be differences in details. For example, in the cited paper you state that BOTH patients showed clinical signs compatible with neurobrucellosis; however, in this MS Case #2 had the organism isolated from blood and there is no mention of CSF nor clinical findings suggestive of CNS involvement other than headache and disorientation that are non-specific findings.

As stated before, we committed a mistake in our previous Emrg Infect. Dis manuscript. The clinical report performed at the hospital, described case number 2 as a presumptive neurobrucellosis; however, we have verified that in case number 2 no CSF culture was performed whatsoever. Therefore, the clinical report described in the present manuscript is correct. Although, this mistake does not change the interpretation of our results, we will submit a note to the J. Emerg. Dis. clarifying this. We sincerely apologize for this error.

2) The crux of the papers, and the only original finding is human infection with B. neotomae; an organism previously considered to be non-pathogenic for humans since no cases had been identified. The identification is based on complex molecular techniques, none of which is detailed in the MS, but is detailed very convincingly in the EID publication. Without that reference one is left wondering how you made this diagnosis. Some comment regarding the reproducibility and predictive values of the techniques used might be useful.

We agree with the reviewer’s comment. We have made obvious in the first sentence of the discussion, our previous contribution. Page X, line Y. We have also included a sentence regarding reproducibility and predictive values of the techniques in the Discussion section. Page X, line Y.

3) In Background section, you mention rare human cases of B. inopinata. You might also mention rare human cases of marine mammal brucella sp. It is interesting that most of these cases have relied on molecular methods to make the diagnosis despite, in many cases no epidemiologic information to explain the source or method of transmission from animal to human.

According to the reviewer’s comment. a sentence mentioning the isolation of Brucella marine-like strains in humans and the lack of epidemiological link has been included in the introduction. Page X, line Y.
4) The MS needs editorial attention regarding English usage and spelling. For example, also in Background (line 56) should read "virulence" arsenal not "virulent". Also (line 63-66) is a run-on sentence.

The usage of English language has been revised thoroughly, including the sentences indicated by the reviewer.

5) Case 1 (line 78) you state that "after hospitalization the patient showed some improvement....did the patient receive any antibiotics during that first admission?"

Yes, the patient was treated with antibiotics. This has been stated in the description of Case 1. Page 3, lines 67-68.

6) Case 1 (line 80-83) Initial blood cultures were negative after 3 days (in view of the difficulty growing brucellae in vitro) were these cultures maintained for longer than 3 days? Also you state that blood culture was repeated and became positive AFTER 3 DAYS; what blood culture system was used?

The sentence has been rephrased and the culture system described. Page 4, lines 82-90.

7) Case 1 (line 89) doxycycline and streptomycin were administered and you state they were administered for 4 weeks and 12 weeks respectively (should this not be the reverse?)

The reviewer’s comment is correct. The sentence has been corrected. Page X, line Y. We apologize for the mistake.

8) Case 1 (line 93 and line 115) please define MLVA16.

It was properly defined under the “abbreviations” section. Page 8, line 184

9) Case 2 (line 107) you state (in both cases) that Rose Bengal agglutinations were positive. This is a rapid screening test but should be confirmed by serum agglutination. Was this done?

Roses Bengal test is more specific and sensitive that regular plate agglutination or card test. This is the most reliable presumptive assay. Therefore, no confirmation by other serological techniques is necessary. See Díaz et al., PLoS Negl Trop Dis 5: e950.

10) Case 2 (line 107) you state that brucellae were isolated from blood but in the previous publication you state it was from CSF. Which is correct and why the confusion?
As stated before this was a mistake. In the previous manuscript, describing the genomic characteristics of the isolate, we made an involuntary mistake. The clinical report performed at the hospital, described case number 2 as a presumptive neurobrucellosis; however, we have verified that in case number 2 no CSF culture was performed whatsoever. Therefore, the clinical report described in the present manuscript is correct. Although, this mistake does not change the interpretation of our results, we will submit a note to the J. Emerg. Dis. clarifying this. We sincerely apologize for this error committed in our previous paper.

11) Discussion (line 142) you mention the need for culture of CSF; was brucella serology performed on the CSF? The presence of brucella antibodies in CSF can be confirmative of neurobrucellosis.

No, it was not performed. However, with the isolation of Brucella from the CSF, and the characteristics of the CSF, neurobrucellosis was confirmed.

12) Discussion (line 145) you imply that the organism retained susceptibility to doxycycline, aminoglycoside and rifampin based on clinical response; were in vitro sensitivity studies performed on the organisms?

Yes, sensitivity tests for antibiotics were performed. This has been stated in page 4, lines 92-93; page 5, lines 114-115.

13) If desert wood rats are not found in Costa Rica but you propose that other rat species may be a reservoir for brucellae, are there data to support this hypothesis? And if so, how do you propose that the patients contracted the infection?

The statement was not constructed as such and we did not intend to put forward a hypothesis. We just call the attention on the presence of other Neotomines in Costa Rica, that in absence of wood rats may play the same role. We do not have a clue how the patients contracted the infection. This was clearly stated in Discussion section Page 7, lines 160-165.