Author’s response to reviews

Title: Molecular characterization of Histoplasma capsulatum isolated from an outbreak in treasure hunters

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Author’s response to reviews: see over
Dear Dr. Kaiser, we have revised and corrected the paper. I hope the changes are correct. Below you will find the changes and corrections made.

First. We will send you by email, the signed consent letter from the patients (to the address you told me in your email).

Second. We have included a “competing interests” in the section between the conclusions and Authors contribution. There are not any acknowledgements.

Third. We have made all the changes that were told by the reviewers.

Reviewer: Mary Brandt
Reviewer's report:
Molecular typing demonstrates that the two strains typed were indistinguishable, but the specificity of the RAPD test is not escribed. The strains appear to be placed in the Lam A clade described by Kasuga, although the confirmatory testing was not performed.

1. We have made the correction as the reviewer indicates.

2. The paper can be cut to a brief report; Figures 1, 2, and 3 can be removed as they do not reveal particular novelty about the disease, the diagnosis, or the epidemiology. Fig 4 can be merged with Fig 5 to use the RAPD portion with all DNAs on one gel.

We have made the correction as the reviewer indicates. The figures 1, 2 and 3 were eliminated and Figure 4 was merged with fig 5.

Other comments:
(1) Background: The mycelial form is not the infectious phase. The infectious form is the microconidium.

We have made the correction as the reviewer indicates. This observation was corrected. Background line 5.
(2) The three-variety taxonomy is now outdated. There are at least eight clades as described by Kasuga. A statement such as “H. capsulatum historically had three varieties…” should be made stating that the three-variety taxonomy is no longer used, and then the reader should be briefly told the current taxonomy.

We have made the correction as the reviewer indicates. Background line 6.

(3) Line 17: The term “histoplasmosis capsulati” is no longer used and can be Removed.

We have made the correction as the reviewer indicates. The term was removed.

(4) Is this really an “epidemic” with six cases? According with Health Statistics (morbidity and mortality of the local Heal Services) there are not cases (neither defunctions) previously registered. The definition of an outbreak is the occurrence of one or more cases of the pathology (histoplasmosis) linked between them (two linked cases of a rare infectious disease may be sufficient to constitute an outbreak). This study is about 6 persons that not live in the zone, they are linked because their source of the infection was the same: the exposure to contaminated dirt during the excavation in the interior of the house at the Tamarindo.

(5) Methods: please describe the serologic tests that were performed or give literature references. The reader may not be familiar with the type of ELISA and capillary testing performed in Mexico.

We added the references requested

(6) “Sensitivity to histoplasmin” does this mean that a skin test was placed?

In material and methods we have mentioned this test in the paragraph: Isolation and identification of *H. capsulatum*.

(7) PCR protocol: was the annealing in the 35 cycles also done at 72C?

We have made the correction as the reviewer indicates. We corrected this observation.

(8) Results: what is meant by “the …showed the fungus to be in its parasitic stage.” In tissue, the fungus demonstrates the yeast phase of growth, and this term should be used.

In Results in paragraph “Isolation and Identification of *H. capsulatum*”, we have mentioned this observation.

(9) Page 10: H. capsulatum var. capsulatum is an invalid name and is no longer used.
We have eliminated this term.

(10) “The serologic tests were positive…” please describe the criteria for a positive test.

We have made the correction as the reviewer indicates. Now in the paragraph “Isolation and Identification of H. capsulatum”, we described the criteria used to give a positive test.

(11) What is the relationship between the RAPD results shown here and the Kasuga clades? Can these isolates be placed into one of the Kasuga-defined clades? If so these data should be provided in the Results.

Molecular typification of the two isolates from this outbreak was compared with the polymorphic DNA patterns of strains collected from VZ-neighboring areas. EVP and LFD isolates presented 100% similarity to the EH-53 HG strain (Figure 2) which was isolated in 1977; therefore, the two isolates were considered as the same strain. The EH-53 strain is included in the Lam A clade (forming a monophyletic group for Latin America) according to Kasanuga et al. [8]; although these isolates were not characterized by four genes as utilized by Kasuga, these isolates could be included in the Lam A clade.

(12) Discussion: “…the patients were infected with the same strain…” How reliable is the RAPD at distinguishing among strains of close and of moderate genetic relatedness?

Yes, the RAPD is a reliable technique to identify the source of infection if they are reproducible results, as in our case.

(13) The manuscript requires revision for English grammar and spelling Throughout

A professional has reviewed the English grammar and spelling

(14) The patients should be more thoroughly described. Were they residents of the Veracruz area, or did they simply visit the area to conduct the digging activities? The fact that two children were involved should also be noted. Their symptoms involved nausea and vomiting, which is somewhat unusual for histopalsmosis.

We have resolved this issue the section of materials and methods.

Reviewer: Harish Gugnani
1. Abstract (page 3 of 30) 7 & 8th lines under Methods: In the sentence “The diagnosis was confirmed using staining and immunological techniques, and culture”, it is necessary to specify the medium on which culture was obtained.

We have made the correction as the reviewer indicates. We mentioned the medium on which culture was obtained in the Abstract and in the Methods.

2. Also the first part of the next sentence “H. capsulatum isolates were identified by PCR using” should be modified as follows. “Identification of H. capsulatum isolates was confirmed by using PCR”.

We have made the correction as the reviewer indicates. Instead of “H. capsulatum isolates were identified by PCR using” by “Identification of H. capsulatum isolates was confirmed by using PCR”.

3. I am saying this since normally H. capsulatum isolates can identified by microscopic morphology and its in vitro conversion to yeast form by cultivating on brain-heart infusion agar supplemented, with 0.1% cystine and with or without blood. The authors should also clarify if they achieved in vitro conversion to yeast form. PCR is used for confirmation of the identity, particularly if an isolate is lacking some morphological features e.g. lack of tuberculate macroconidia but not for first attempt in identification.

We don’t achieved in vitro conversion to yeast form.

4. In the last sentence before Conclusion-the sentence” The etiological agent was clearly identified” is superfluous, and it should be deleted.

We deleted this sentence.

5. Background (page 6 of 30) lines 1-3: The sentence “This mycosis may range wide so from being clinically benign to severe, inclusive fatal, depending on the amount of propagules inhaled and the immunological state of the infected host [3]” is linguistically incorrect and should be modified as “The clinical manifestations of this mycosis may range widely from being clinically benign to severe, inclusive fatal, depending on the amount of propagules inhaled and the immunological state of the infected host [3].” The modified sentence would better convey the sense implied.

We have made the correction as the reviewer indicates. The sentence was correct as the reviewer recommend. Instead “This mycosis may range wide so from being clinically benign to severe, inclusive fatal, depending on the amount of propagules inhaled and the immunological state of the infected host [3]” by “The clinical manifestations of this mycosis may range widely from being clinically benign to severe, inclusive fatal, depending on the amount of propagules inhaled and the immunological state of the infected host [3].”

Also in the ending portion of the last sentence, “was carried out phylogeographically locating the molecular pattern with the clades described for Latin America” should be deleted as this information is mentioned in detail under a separate head under Materials and Methods on page 8.
We have made the correction as the reviewer indicates. The sentence was deleted.

Page 7 of 30 Isolation and identification of H. capsulatum:
After the third sentence, it should be mentioned that the samples were cultured on Saboraud dextrose agar with antibiotics (specify) and without antibiotics. The following sentence: In Sabourand cultures (SDA) and SDA with antibiotics, capsulatum isolates were identified using microscopic morphology is linguistically incorrect and convey the wrong sense. It should be corrected as: The isolates were identified on the basis of colonial and microscopic morphology.

We have made the correction as the reviewer indicates. Instead of “In Sabourand cultures (SDA) and SDA with antibiotics”, by “The isolates were identified on the basis of colonial and microscopic morphology.”

capsulatum isolates were identified using microscopic morphology is by
Further if they achieved in vitro conversion of the isolates to yeast form, it should be so mentioned.

The in vitro conversion of the isolates to yeast form was not performed.

5th and 7th line: The phrase “The serology tests were done in search” should be shortened as ‘The serological tests’

We have made the correction as the reviewer indicates. Instead of “The serology tests done in search” by “The serological tests”.

Conclusion-The sentence ” The etiological agent was clearly identified” is superfluous as it is clear from the subsequent write-up

The sentence was deleted.

Materials and Methods
Under the section, Molecular identification and characterization of the isolates (page 8 of 30), the first sentence “The two isolates were identified using PCR. should be changed to “Molecular identification of the two isolates was done using PCR.”

We have made the correction as the reviewer indicates. Instead of “The two isolates were identified using PCR”, by “Molecular identification of the two isolates was done using PCR”.

Further I would prefer the phrase “Molecular typing” rather than “Molecular identification”

We have made the correction as the reviewer indicates. Instead of “Molecular identification” by “Molecular typing”
Under the section “DNA Extraction, the word “achieved” in the sentence should be replaced by “obtained”.

We have made the correction as the reviewer indicates. The word “achieved” was replaced by “Obtained”.

Results
The subhead “Identification and Molecular Characterization of the Isolates” (page 10 of 30) has to be changed to “Molecular Characterization of the Isolates”.

We have made the correction on the subhead as the reviewer indicates, instead of “Identification and Molecular Characterization of the Isolates” by “Molecular Characterization of the Isolates”.

Further the details of the method used for molecular typing already described under Materials and Methods should be avoided. Only the results of the molecular typing need to be mentioned.

We have made the correction as the reviewer indicates.

Discussion. In the first paragraph (page 12 of 30): in the 2nd sentence, the word “writes” and “is” in the 7th line of this paragraph should be changed to “wrote and” was respectively.

Instead of “write” and “is” by “wrote” and “was”.

Also in the second paragraph in the same page, in the second sentence, the phrase “The histoplasmosis epidemiological outbreaks have been reported” should be modified as “The outbreaks of histoplasmosis have reported”. Further in the next sentence, the word “epidemic” should be deleted as it is superfluous.

We have made the correction as the reviewer indicates. We changed the phrase “The histoplasmosis epidemiological outbreaks have been reported” by “The outbreaks of histoplasmosis. And we have deleted the word “epidemic”.

Also the word “epidmiiiiological” before “outbreak” should be deleted wherever it occurs in the manuscript, as it superfluous.

We have made the correction as the reviewer indicates. We have deleted the word “epidemiological” before “outbreak” in all manuscript.

In the sixth paragraph of the discussion (page 14 of 30), the authors write “For example, sick bats can act as dispersers of the parasite incorporating the fungus into new favorable environments possibly through cadavers [12].” What do the authors mean by ‘sick bats’, sick from what. Do bats really become clinically ill with h. capsulatum infection. Though H. capsulatum has been
recovered from their internal organs, viz. live, spleen but in almost all the cases without any lesions on the organs. So I strongly suggest that the words ‘sick bats’ should be replaced by “infected bats”.

We have made the change as the reviewer indicates, instead of “sick bats” by “infected bats”.

In the last paragraph (page 14 of 30), while talking of therapeutic scheme, the authors write “All of the patients evolved favorably” Use of the word “evolved” is confusing as well as amusing. Probably what the wanted to say that the symptoms and lesions resolved. If so, the sentence would need to be modified as “The symptoms and lesions in all the patients resolved.” However, the regimen and duration of the therapy would need to be mentioned.

We have made the correction as the reviewer indicates, instead of “All of the patients evolved favorably” by “The symptoms and lesions in all the patients resolved.” And we mentioned the regimen and duration of the therapy.

Conclusion, “The isolates could be considering in the same clade too, the word considering should be replaced by “considered” (correct grammar).”

We have made the correction as the reviewer indicates, instead of “considering” by “considered.”

Competing interests
Under this section, the word “There’ should be replaced by “The authors”

We have made the correction as the reviewer indicates, instead of “There” by “The authors”.

Thank you for your interest.

Sincerely

Ma. Eugenia Manjarrez Zavala