Reviewer's report

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

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Reviewer: Mary Brandt

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This paper describes a cluster of six histoplasmosis infections in Veracruz, Mexico, a known endemic region for this disease. The disease was diagnosed using conventional methods. Molecular typing demonstrates that the two strains typed were indistinguishable, but the specificity of the RAPD test is not described. The strains appear to be placed in the Lam A clade described by Kasuga, although the confirmatory testing was not performed. The authors do not describe any particular novelty about this cluster or the laboratory findings. 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.

Other comments:

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

(2) The three-variety taxonomy is now outdated.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.

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

(4) Is this really an “epidemic” with six cases?

(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.

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

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

(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.

(9) Page 10: H. capsulatum var. capsulatum is an invalid name and is no longer used.

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

(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? How likely is it that the results are actually due to homoplasy?

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

(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.