Author response to reviewer comments:

We would like to thank the reviewers for their insightful comments. We believe that the new version of the manuscript, including the referee’s suggestions, improved our contribution. Please find below a point by point reply to the reviewers. We copied the referee’s comments in grey and our reply is in black.

The authors present a case report about a 57 year old female Venezuelan immigrant with paracoccidioidomycosis. The study is relevant, once only few cases of paracoccidioidomycosis have been reported in Europe, where it is considered a rare imported infection. The manuscript is well written and the case well presented. However, some additional information and minor corrections may improve the final version of the manuscript. It is recommended that the authors include in their manuscript the following information:

Abstract

# In the sentence "Because Paracoccidioides sp. are extremely slow growing in vitro, direct observation of fungal elements in histopathology and immunohistochemistry as well as molecular diagnostics are often key to diagnosis." is important add information about direct microscopic examination and serological tests. In general, direct microscopic examination of clinical specimens is the gold standard for PCM diagnosis. In addition, specific serological tests are important in not only the diagnosis of PCM, but also in the assessment of host response to specific treatments.

We agree with the reviewer. In the discussion of the new version of the manuscript, we consider the diagnostic gold standard for PCM, histomorphology and the use of auxiliary diagnostic techniques (see page 2, paragraph 1). We also added a reference of Shikanai-Yasuda et al (reference 23 in our manuscript) to illustrate the possibility of false-negative serology results.

Clinical observations

# We suggest rewriting the sentence "Differentiation from histoplasmosis appeared impossible by light microscopy." The Paracoccidioides sp. structure is characterized by Multiple budding yeast form whereas that of simple budding Histoplasma sp.

While we agree with the reviewer that multipolar budding can be indicative of PCM, several reviews (e.g. Guarner et al, reference 21 in our manuscript) and our own experience with this case suggest that this growth pattern can be absent in PCM. In our case, initial slides showed only small yeasts, but staining of additional slides led the discovery of yeast forms suggestive of PCM. The sentence has been rewritten to clarify this point. (see page 3, paragraph 2) We also changed the order of paragraph 3 on this page and paragraph 1 on page 4 to make the diagnostics aspect of the Clinical observations easier to understand.

# "Under the differential diagnosis of a disseminated fungal infection, we initiated a therapy using liposomal amphotericin B." The drug dosage information is important information and should be inserted in the text. Please note this information throughout the session.

We thank the reviewer for raising this point and we added the information on drug dosage throughout the manuscript (see page 4, paragraph 2).
# Why the only serological test performed for paracoccidioidomycosis was ID? Why was the complement fixation test not performed?

We agree with the reviewer that these tests are relevant, but, unfortunately, Paracoccidioidomycosis complement fixation is not available at our lab, nor at others in Germany.

# "Histoplasma and Paracoccidioides antibodies were detected by ID." The information of titer of circulating antibodies against Paracoccidioides and Histoplasma are available?

Again, we agree with the reviewer but, the immunodiffusion titers were not quantified for Paracoccidioides and Histoplasma in our diagnostic laboratory. The complement fixation titer for Histoplasma was negative as described in the manuscript (see page 4, paragraph 1).

# Are the methodologies employed sufficient to state that it is an infection caused by Paracoccidioides brasiliensis? Otherwise, I suggest rewriting the conclusion in the last paragraph of section Clinical observations. As suggestions: "In conclusion, the patient had Paracoccidioides sp. infection as suggested by..." or "In conclusion, the patient had paracoccidioidomycosis as suggested by..."

We agree with the suggestion and have rewritten the conclusion. The methodologies we used allow us to confidently differentiate between Paracoccidioides brasiliensis and other members of the Paracoccidioides family, i.e. Paracoccidioides lutzii. However, we can't differentiate between P. brasiliensis and some of the recently described Paracoccidioides species that were previously thought of as subtypes of P. brasiliensis (Turissini et al, 2017).

Discussion

# "In endemic countries, the mortality of PCM has been estimated between 6.1-7.6%." The references cited deal with Brazilian studies. Would it be possible to enter information from other countries where paracoccidioidomycosis is endemic?

We agree that the epidemiology of PCM outside Brazil is an important point to consider. However, the only information we could find regarding lethality of PCM outside Brazil dates from the 80s (Negroni et al, reference 9). This information and reference have been added to the manuscript. As far as we know, more recent data on PCM mortality rates are only available for Brazil. However, we added information on the incidence rates in other endemic countries.

# Is there any reported case of co-infection between paracoccidioidomycosis and sarcoidosis? This information is relevant to the discussion.

While paracoccidioidomycosis can resemble sarcoidosis (as in the excellent additional reference the reviewer suggested), there is, to our knowledge, no described case of concomitant sarcoidosis and PCM. This has been elaborated in the discussion (see page 6, paragraph 1).

# "PCM can be diagnosed either via histological identification of typical fungal elements in tissue or growth of Paracoccidioides in fungal culture of patient samples, displaying a multibudded “pilots wheel” configuration in microscopy." In this case, “histological identification” is the same that direct examination by microscopy? This is not clear to me. Please check this information.

We thank the reviewer for this comment. In the case we present, yeasts were found microscopically both in tissue samples (histology) and the bronchioalveolar lavage (direct observation). In both, we observed multiple buds, but not the “pilot’s wheel” configuration. We modified the manuscript to
clarify this point in the “Clinical observations” portion (see page 3, paragraph 3) and expanded the segment concerning the presentation of paracoccidioidomycosis in microscopy. (page 6, paragraph 3 and page 7, paragraph 1)

# In the discussion session, I suggest an approach about serological testing. In many cases, where it is not possible to isolate the fungus in culture or histopathological tests, serological tests are great tools that aid in the diagnosis of paracoccidioidomycosis.

We thank the reviewer for this suggestion. We added a paragraph on antibody detection including benefits and limitations in (see page 7, paragraph 2).

Figure

# Figure 1-H The authors wrote: "... showing an exophytic fungal growth ..." However, what is seen is not the fungal growth, but the exophytic lesion. We suggest you should rewrite such as "...showing an exophytic lesion ...".

Thank you, this has been clarified in the figure description.

References

# The references number 7 and 16 are identical. Please, remove one of the two references. Also check this information at end of section Clinical observations.

Thank you, the error has been corrected.

# The reference "Paracoccidioidomycosis with sarcoid-like lesions: a diagnostic challenge" (PMID: 28562770) it seems interesting to enrich your work.

Thank you for pointing this out to us, this reference is now included in our manuscript.