

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([see an example](#)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Mansonella ozzardi corneal lesions in the Amazon: a cross-sectional study
<b>AUTHORS</b>	Vianna, Lucas; Martins, Marilaine; Cohen, Jacob; Cohen, Marcos; Belfort Junior, Rubens

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Luís Marcelo Aranha Camargo Professor, Medical Doctor, PhD University of São Paulo In head of the Rondônia Advanced Research Unit-Amazonia-Brazil  No conflict of interests
<b>REVIEW RETURNED</b>	19-May-2012

<b>THE STUDY</b>	1-) No statistical method described or used to detect statistical correlation between microfilaria x ocular lesions 2-)The sensitivity of the blood thick smear examination is not reliable. There maybe some patients with microfilaria in the non affected group. They should better use the blood filtration with polycarbonate membrane plus microscopic examination with Giemsa stain and/or PCR for microfilaria detection in all blood samples. 3-) It is not clear where the PCR was used
<b>RESULTS &amp; CONCLUSIONS</b>	The sensitivity of the blood thick smear examination is not reliable. There maybe some patients with microfilaria in the non affected group. They should better use the blood filtration with polycarbonate membrane (3-5 micra diameter) plus microscopic examination with Giemsa stain and/or PCR for blood examination. The absence of a control group also affects the reliability of the study.
<b>GENERAL COMMENTS</b>	It's an original article and aims to produce more information about clinical manifestations of a high prevalent neglected Amazonian disease. Unfortunately the methodology is biased.

<b>REVIEWER</b>	Dr Fatima Kyari Research Degree Fellow International Centre for Eye Health (ICEH), London School of Hygiene and Tropical Medicine (LSHTM), London, United Kingdom  I have no competing interests.
<b>REVIEW RETURNED</b>	11-Jun-2012

<b>THE STUDY</b>	1. The term "suspicious corneal lesions" frequently used needs to be described/defined. 2. How did the investigators distinguish between corneal lesions due
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	<p>to M. ozzardi and those from other causes?</p> <p>3. Tables 1, 2 and 3 showed associations between the presence of microfilaraemia and corneal lesions assessed by the different examinations. However, no statistical tests were described to give the significance of association.</p> <p>4. The standard of written English is acceptable. However, there are some minor corrections needed.</p> <p>page 6, line 11 ...in humans, M.ozzardi BEING one of them. The first description....</p> <p>page 7, line 11 .. al patients had THEIR eyes examined..</p> <p>page 7, line 25 ...patients with suspicious corneal lesions at the eye examination were SUBJECTED to biomicroscopy..</p> <p>page 7, line 29 THE CCME...</p> <p>page 10, line 42 use "prevalence", and not "prevalence rate"</p> <p>page 10, line 48 ...but there is NO onchocerciasis in THOSE parts of Brazil..</p> <p>page 11, line 6 ... onchocerca EXIST.</p> <p>page 12, line 34 ... eyes with no BIOMICROSCOPIC lesions..</p>
<b>REPORTING &amp; ETHICS</b>	With the information provided and manuscript, I am not able to make a definite statement on plagiarism, fabrication, and undeclared conflict of interests

<b>REVIEWER</b>	Thomas B. Nutman, MD Head, Helminth Immunology Section Laboratory of Parasitic Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health Bethesda, MD 20815
<b>REVIEW RETURNED</b>	14-Jul-2012

<b>THE STUDY</b>	<p>1) What is actually missing here are data demonstrating the statistical validity of their findings. The authors need to spell out the types of statistical methods they will perform and then actually perform them on their data. I actually performed statistics on the data presented in Tables 1-3; for only data in Table 1 was their a statistically significant finding.</p> <p>2) Given that the authors are using PCR in corroborate their microscopy data, the details of this must be performed. Also, given that biopsies were performed in 2 patients, PCR should be applied to the tissue in order to demonstrate definitively the relationship between the ocular disease and the presence of microfilariae in the eye proper.</p> <p>3) A native English speaker should edit the manuscript to make the English a bit more colloquial and correct a few mistakes in syntax</p>
<b>RESULTS &amp; CONCLUSIONS</b>	Both the statistical validity of their findings and a definitive speciation of the ocular microfilariae would make this much more compelling, particularly because of number of the pictures of microfilariae (particularly the ones with the forked tail) are not characteristic of

	Mansonella ozzardi.
<b>REPORTING &amp; ETHICS</b>	There is no statement about ethical approval nor about informed consent in the manuscript.

### VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Luís Marcelo Aranha Camargo  
Professor, Medical Doctor, PhD  
University of São Paulo  
In head of the Rondônia Advanced Research Unit-Amazonia-Brazil

1) "No statistical method described or used to detect statistical correlation between microfilaremia x ocular lesions."

R: We are sorry for this mistake. Statistical analyzes were included in the text (highlighted).

2) "The sensitivity of the blood thick smear examination is not reliable. There maybe some patients with microfilaremia in the non affected group. They should better use the blood filtration with polycarbonate membrane plus microscopic examination with Giemsa stain and/or PCR for microfilaremia detection in all blood samples."

R: We were really not clear in our methods section. In all patients thick smear, Knott and polycarbonate membrane filtration and PCR were performed by the co-author Marilaine Martins. This information was added to the text.

3) "It is not clear where the PCR was used."

R: PCR was used to confirm if the species of microfilariae were Mansonella ozzardi in peripheral blood of all the patients.

4) "The absence of a control group also affects the reliability of the study." R: We performed confocal microscopy in both eyes of all the 22 patients with keratitis. All of them had unilateral keratitis. We observed that the non affected fellow eye never showed alterations in the confocal microscopy.

5) "It's an original article and aims to produce more information about clinical manifestations of a high prevalent neglected Amazonian disease. Unfortunately the methodology is biased."

R: This is mainly due to conditions of the work field. We visited several coastal communities (distant travel hours or days of each other), traveling by boat and examination of patients in such places were very difficult because they had to come aboard for this.

Reviewer 2: Dr Fatima Kyari  
Research Degree Fellow  
International Centre for Eye Health (ICEH),  
London School of Hygiene and Tropical Medicine (LSHTM)  
London, United Kingdom

I have no competing interests.

1) "The term "suspicious corneal lesions" frequently used needs to be described/defined."

R: We call it "suspicious corneal lesions" the ones similar to those described in previous articles cited in our references. It is, basically, nummular keratitis. The description and this information were added to the revised text.

2) "How did the investigators distinguish between corneal lesions due to *M. ozzardi* and those from other causes?"

R: In the original text we wrote in the Results section "The clinical characteristics of the lesions as well as the high prevalence exclude the possibility of traumas and other known causes for the differential diagnosis, specifically Onchocercosis." and in the Discussion section "A comparison with other filarial diseases shows some similarities with onchocerciasis but there is no onchocerciasis in those parts of Brazil and also the clinical picture is different. Onchocerciasis has been excluded by the characteristics of the microfilaria tail and because it exists in Brazil only close to the Venezuela border. None of the patients lived or visited areas where *Onchocerca* exist." Besides this, until today, onchocercosis is limited to the region of the Yanomami Indians. (The Onchocerciasis Elimination Program Sauerbrey for the Americas (OEPA) M. Annals of Tropical Medicine & Parasitology, Vol 102, Supplement No. 1, S25-S29 (2008). This information was added to the text.

3) "Tables 1, 2 and 3 showed associations between the presence of microfilaremia and corneal lesions assessed by the different examinations. However, no statistical tests were described to give the significance of association."

R: Statistical analyzes were included in the text (highlighted).

4) "The standard of written English is acceptable. However, there are some minor corrections needed."

R: We apologize. All suggested changes listed above were made in the review.

page 6, line 11 ...in humans, *M.ozzardi* BEING one of them. The first description....

page 7, line 11 .. al patients had THEIR eyes examined..

page 7, line 25 ...patients with suspicious corneal lesions at the eye examination were SUBJECTED to biomicroscopy..

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page 10, line 48 ...but there is NO onchocerciasis in THOSE parts of Brazil..

page 11, line 6 ... onchocerca EXIST.

page 12, line 34 ... eyes with no BIOMICROSCOPIC lesions..

5) "With the information provided and manuscript, I am not able to make a definite statement on plagiarism, fabrication, and undeclared conflict of interests."

R: A declaration of no competing interests was made in Methods Section.

Reviewer 3: Thomas B. Nutman, MD  
Head, Helminth Immunology Section  
Laboratory of Parasitic Diseases  
National Institute of Allergy and Infectious Diseases  
National Institutes of Health

1) "What is actually missing here are data demonstrating the statistical validity of their findings. The authors need to spell out the types of statistical methods they will perform and then actually perform

them on their data. I actually performed statistics on the data presented in Tables 1-3; for only data in Table 1 was their a statistically significant finding.”

R: Statistical analyzes were included in the text (highlighted).

2) “Given that the authors are using PCR in corroborate their microscopy data, the details of this must be performed. Also, given that biopsies were performed in 2 patients, PCR should be applied to the tissue in order to demonstrate definitively the relationship between the ocular disease and the presence of microfilariae in the eye proper.”

R: The PCR was not applied to the ocular tissue because it was not available. It will be a part of our next study.

3) “A native English speaker should edit the manuscript to make the English a bit more colloquial and correct a few mistakes in syntax.”

R: We revised it again including the suggestions made by one of the reviewers.

4) “As mentioned above, both the statistical validity of their findings and a definitive speciation of the ocular microfilariae would make this much more compelling, particularly because of number of the pictures of microfilariae (particularly the ones with the forked tail) are not characteristic of Mansonella ozzardi.”

R: Statistical analyzes were included in the text (highlighted). The ideal confirmation of the presence and what kind of microfilaria would be through corneal biopsy, but this is difficult, especially in the case of asymptomatic patients. This will be the subject of future studies. The intention of the article is to produce more evidence of the association between microfilaraemia by M. ozzardi and lion eye.

5) "There is no statement about ethical approval nor about informed consent in the manuscript."

R: The following statement was already in the Results, but now its in the Methods section: “The study was prospective approved by the UNIFESP Ethical Committee. All participants signed the protocol’s informed consent.”

#### VERSION 2 – REVIEW

<b>REVIEWER</b>	Prof. Luís Marcelo Aranha Camargo MD, Phd University of São Paulo-Brazil In Head of the Rondônia Research Unit
<b>REVIEW RETURNED</b>	29-Sep-2012

<b>THE STUDY</b>	<p>1-)Page 3, fFrom line 12 to 17 the author should break the text in order to improve the text.</p> <p>2-) There are no exclusion or inclusion criteria</p> <p>3-) It is not clear in the tables if the "microfilaremia" was detected by microscopy and/or Knot and/or membrane filtration and/or PCR.</p> <p>4-)Page 3, Line 46= separate the geographica coordinates with comma</p> <p>5-) In tbales 2 and 3: the author shuold stress that there is ststistically significance in the association ( p=0.1347 and p=0.4266))</p> <p>6-)In the discussion the author should stress that there is no association between the lesions and "microfilaremia". It is not clear and may confound the reader.</p>
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<b>REPORTING &amp; ETHICS</b>	i suggest to put the number of the UNIFESP ethical clearance number
<b>GENERAL COMMENTS</b>	<p>New bibliography sugested Genetic Characterization of Atypical Mansonella (Mansonella) ozzardi Microfilariae in Human Blood Samples from Northeastern Peru.</p> <p>Marcos LA, Arrospide N, Recuenco S, Cabezas C, Weil GJ, Fischer PU. Am J Trop Med Hyg. 2012 Sep;87(3):491-4. Epub 2012 Jul 23. Contribution of the PCR assay to the diagnosis of Mansonella ozzardi in endemic areas of Argentina].</p> <p>Degese MF, Cabrera MG, Krivokapich SJ, Irazu LE, Rodríguez MA, Guarnera EA. Rev Argent Microbiol. 2012 Apr-Jun;44(2):97-100. Spanish.</p> <p>11.</p> <p>Investigation of the occurrence of Mansonella ozzardi in the State of Rondônia, Western Amazonia, Brazil.</p> <p>Basano Sde A, Camargo Jde S, Vera LJ, Velasques SN, Ogawa GM, Medeiros JF, Fontes G, Camargo LM. Rev Soc Bras Med Trop. 2011 Oct;44(5):600-3. Epub 2011 Aug 26. PMID: 21877064 [PubMed - indexed for MEDLINE] Free Article Related citations</p> <p>12.</p> <p>Improvement of a PCR test to diagnose infection by Mansonella ozzardi.</p> <p>Vera LJ, Basano Sde A, Camargo Jde S, França AK, Ferreira Rde G, Casseb AA, Medeiros JF, Fontes G, Camargo LM. Rev Soc Bras Med Trop. 2011 May-Jun;44(3):380-2.</p> <p>Nested PCR to detect and distinguish the sympatric filarial species Onchocerca volvulus, Mansonella ozzardi and Mansonella perstans in the Amazon Region.</p> <p>Tang TH, López-Vélez R, Lanza M, Shelley AJ, Rubio JM, Luz SL.</p>

**VERSION 2 – AUTHOR RESPONSE**

Answer to reviewer Luís Marcelo Aranha Camargo: We thank you so much, again, to Dr. Camargo for his contribution in our article. All suggested changes, including references suggested, were included in the text, in red.

**VERSION 3 - REVIEW**

<b>REVIEWER</b>	PROFESSOR LUIS MARCELO ARANHA CAMARGO MD, PhD DEPARTMENT OF PARASITOLOGY ICB/USP RESEARCH UNIT-RONDÔNIA-BRAZIL
<b>REVIEW RETURNED</b>	16-Oct-2012

- The reviewer completed the checklist but made no further comments.