Author’s response to reviews

Title: Imported loiasis in France: a retrospective analysis of 167 cases with comparison between sub-Saharan and non sub-Saharan African patients.

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Version: 1 Date: 19 May 2019

Author’s response to reviews:

Dear Editor,

Please find enclosed the revised version of the article titled “Imported Loiasis in France: an analysis of 167 cases with comparison between sub-Saharan and non sub-Saharan African patients”.

We responded point by point to all reviewers' comments (see below). For the majority of them we agreed with these comments and modified the text accordingly. When the two reviewers made the same request for additional information (at three occasions: R1/comment 28 and R2/comment 10; R1 and R2/comment 25; R1/comment 7 and R2/comment 13), we were able to add this data in the revised version. For a limited number of remarks we did not entirely agree with the reviewer's position but proposed a compromise modification in the new version. For 2 comments the requested information is not available in the database. For two requests (R1 / comment 10 and 26) we are not able to do the additional analyses requested since the database manager and statistician who worked on this study are no longer available and not recoverable due to the extended delay between the end of the study and the finalization of the article. Asking new professionals who are not familiar with the study to look into the database is not easy and will require time (and funding). In addition, the extent of the additional work to be done seems disproportionate to us, given the benefits that readers, particularly clinicians who will probably be the main target, will be able to get from it.

I guarantee that all authors approved the final manuscript.
Hoping that you will find this work worthy of being published in BMC Infectious diseases, on behalf of co-authors I address respectful greetings.

Olivier Bouchaud, MD, Ph D

Reviewer 1:
Comment 1: “one may wonder why the authors excluded from their series those patients who presented clinical signs very suggestive of loiasis (Calabar, eyeworm), but were found amicrofilaremic and seronegative at lab examinations. It is well known that many loiasis patients have an occult infection (without blood mf) and that the serological tests are not perfect in terms of sensitivity (as shown in the paper). It would be interesting to get an idea of the number of these excluded patients.”

this comment is relevant but in fact we did not exclude patients with clinical signs suggestive of loiasis but found amicrofilaremic and seronegative because because our “gateway” for this study was only a parasitological diagnosis of loiasis as stated in the materials and methods section (“All the patients with a parasitological diagnosis of loiasis (microfilaremia >1/ml and/or serologic tests) were selected”). It was a choice to have the maximum chance of identifying with certainty the loiasis seen in the participating hospitals. Of course, some parasitologically negative loiasis probably have escaped us and it is impossible for us to know how many, but considering that the risk that a loiasis is both serologically and microscopically negative is low, the number of loiasis lost because of our inclusion criteria is probably very limited.

Comment 2: Line 59: reference 13 corresponds to a series of 186 subjects in the US, not in the UK or Italy

we totally agree with the comment. It is a mistake. The first two studies took place in the United Kingdom and Italy and the one with 186 inclusions in the United States. We modified the manuscript as follows: “The three largest studies including 100 cases for two of them and 186 for the third one, took place in England, Italy and the United States, respectively”

Comment 3: Line 62: replace "dyethylcarbamazine" by "diethylcarbamazine (DEC)"; and use the abbreviation DEC throughout the text.

we agree with the reviewer's suggestion and have modified the text accordingly.

Comment 4: Line 64: are these encephalopathies really due to "parasite lysis"? See Wanji et al., PLoS Negl Trop Dis. 2017 Jul 7;11(7):e0005576

the reviewer raises the important issue of the pathophysiology of severe adverse events after treatment, including encephalopathy. It refers to a study on monkeys that, without contradicting conventional data on the subject, brings new elements by suggesting that the primary pathological event following ivermectin treatment is a vasculopathy associated with intra-vascular microfilarial destruction within smaller blood vessels that damages them and leads to changes in the tissues supplied by these vessels. Since these data are preliminary, made on an animal model, although very similar to humans, and do not really contradict the classic hypothesis mentioned in the article, it seems to us that we do not have enough robust elements to change the meaning of this sentence. However, we proposed to add “classically assigned to parasite lysis” and, as indicated in response to comment 15, we have deleted in the methodology section the reference to parasitic lysis in the revised version, indicating “post-treatment reaction” instead of “Post-treatment parasitic lysis reaction”. We did the same in Table 4 and in the discussion.

Comment 5: Line 71: why were the cases restricted to this period, as we are now in 2019?

we agree that the delay between the collection of data completed at the end of 2013 and the presentation of the results is too long. It is simply explained by the “alea” of the real life ! The young clinician in charge of the study dropped the work being written for personal reasons. The search for a
successor was not simple, leading the head of the study to take over the work himself despite a heavy load.

Comment 6 : Line 74: were there cases with a microfilaraemia <1/mL?
- the authors are not sure to fully understand the reviewer's remark. Line 74 gives the inclusion criteria as mentioned above (positive microfilaremia i.e. >1/ml and/or positive serologic tests)

Comment 7 : Line 94: What volume of blood was used to prepare the blood smear? In case of negative blood smear, were concentration techniques applied?
- the observation under the microscope was made on a drop of fresh blood between slide and slip cover. If microfilariae were visualized, precise identification and counting were done on a 15 microliter thick film after staining. In case of negative microscopic examination, leucoconcentration on 5 mL was performed. These clarifications have been added in the text.

Comment 8 : Lines 97-102: could the authors add numbers, or (i), (ii), (iii) etc. to better explain the two techniques used in a given parasitology department?
- the techniques are actually diverse and differently associated according to the laboratories. In the revised manuscript we added clarifications on the associations (one or two screening tests, and in case of positivity, followed by one or two confirmation techniques) and we grouped the combinations (screening + confirmation) into 3 groups identified from i to iii.

Comment 9 : Line 103: were all the techniques really "home-made" techniques? What is the ELISA mentioned at line 99?
- at the time of the study's preparation in 1992, participating parasitologists described their techniques as "homemade". Apart from the ELISA tests, which were commercial kits (we added that precision in the revised version), it is likely that during the course of the study, some techniques have evolved or have been replaced by commercial serologies but we do not have the details of these possible changes and it will be very difficult to obtain them, the study's being rolled out over 20 years.

Comment 10 : Line 104: the various techniques should be described in a supplementary information file; and the number of subjects tested with each of them should be presented.
- it is indeed possible to specify the detail of each technique. Except for immunoelectrophoresis for which we have the denominator (data regarding the specific arc)(see comment 22), it is also possible in theory to associate the number of subjects included with each technique. However this research will be complex and very long because it would be necessary to ask each centre for this information. Over a period of 20 years the work will be huge knowing that many of the parasitologists in duty at the time of the study are no longer accessible (retired or in another hospital/city). In addition the authors believe that this article is aimed primarily at clinicians, mainly non-specialists in tropical medicine, who need additional information to better manage this rare pathology of return. It is very unlikely that the details of the parasitological techniques will really interest them.

Comment 11 : Line 105: "undetermined when it was not negative but under the threshold of positivity for each technique" is not very clear. Definitions of positivity and negativity should be clarified.
- the "undetermined" result defined in the manuscript as “not negative but under the threshold of positivity for each technique” is the information as described by the laboratory for the patient concerned. In practice for the study we considered these undetermined results as negative. It is true that we should have specified it in the manuscript. The authors have therefore added this clarification to the revised version.

Comment 12 : Line 114: replace diehtylcarbamazine by "diethylcarbamazine" or "DEC"; was DEC given at the same dose to all patients? Was the starting dose adapted to the microfilarial density? What was the duration of a standard course (say this here, even if the information is given below Table 4)? Was DEC systematically given with antihistamines or corticosteroids?
- as stated in comment 3 we have, according to the reviewer's suggestion, systematically replaced diethylcarbamazine by DEC.
- DEC was not given at the same dose to all patients but the different schemes used were very
close and based on the classic recommendations for using DEC (ie progressive dosage). As this was a retrospective study, there could not be any imposed protocol: this is why the dosage (including a starting dose adapted or not to the microfilarial density) was not specified in the methodology. The different observed regimens, however, were summarized in the legend of Table 4. However, in response to the request of the reviewer and as it is a standardized duration, we have specified in the methodology section in the revised version the duration of treatment once the dosage arrived at full dose.

As stated line 202 of the result section “A preventive treatment of parasite lysis reaction (anti-histaminic and/or corticosteroids) was given in 26 patients”, DEC was not systematically given with antihistamines or corticosteroids.

Comment 13: Lines 115 and 116: as some patients were asymptomatic from the beginning, the authors could consider the possibility to replace "disappearance" by "absence"? and "and/or" by "and"?

We agree with the reviewer's suggestion and have modified the text accordingly.

Comment 14: Line 117: what "biological changes" refers to? microfilaremia? Post-treatment serologic tests? Eosinophilia? Replace by "when signs and symptoms persisted and microfilarial levels did not change significantly"?

We agree with the reviewer's suggestion and have modified the text accordingly.

Comment 15: Line 118: "Post-treatment parasitic lysis reaction" is probably incorrect. See comment above.

The authors are aware that this classic data is possibly called into question, at least partially, by the Wanji study of. We deleted “parasitic lysis” to keep only “Post-treatment reaction...”.

Comment 16: Line 136: can the authors confirm that both the median and the lower interquartile values are 3 months? Spell out "IQR" in the text.

We confirm that both the median and the lower interquartile values are 3 months.

We add IQR and spelling in the List of abbreviations.

In Table 1, replace "Republic of Central Africa" by "Central African Republic".

Done.

Comment 17: Line 144: are Ivory Coast, Mali and Rwanda endemic for loiasis? Were there other possible "countries of acquisition" for these subjects?

We discuss this point in the discussion section. These countries are not usually considered endemic but according to some authors, the western part of the African rain forest has been considered in the past as a possible endemic zone for loiasis. Consequently, the theoretical hypothesis can be formulated that transmission may persist at a low level in some remote forested areas of these regions. The same argument can be proposed for Rwanda. However, even if clinicians in charge of patients knowing that these countries were not considered recognized areas of transmission specified that patients had not travelled to other countries considered endemic, it is difficult to propose this hypothesis of persistent transmission as the most likely. The reliability of the patients' data does not allow this and it is necessary to consider as a first hypothesis that these patients have travelled to endemic areas without having reported it. This is one of the well-known limitations of retrospective studies. The authors acknowledge that this "hierarchy" of hypotheses was not clear in the manuscript and have modified the text to clarify it.

Comment 18: Line 150: clarify "symptoms of loiasis". The sentence suggests that pruritus is not a symptom of loiasis.

"symptoms of loiasis" alludes to symptoms of loiasis spontaneously reported by patients, including pruritus. This was the case for 56.8% of patients. For the others, it is in particular during a systematic “return consultation”, without spontaneous clinical complaint of the patients, and whereas the question of a pruritus has been specifically posed, that the diagnosis was evoked. To clarify this point we add to the beginning of the sentence: “Spontaneous reporting of symptoms of loiasis...”.

Comment 19: Line 156: in the Methods section, the authors distinguish Calabar oedemas ("recurrent and short-lasting (less than one week) painless oedema of the extremities") and other oedemas
("subcutaneous oedema with a different location or more prolonged duration"). Do the 29 patients with "subcutaneous migratory oedema" correspond to the latter (definitions are not the same)?

- the 29 patients with "subcutaneous migratory oedema" correspond to the “other oedemas”. To avoid any ambiguity we deleted the word "migratory" in the revised version.

Comment 20 : Lines 160-161: the unit is probably not mm3, but milliliter

- we totally agree with the comment. It is a mistake. We have modified the text accordingly.

Comment 21 : Line 161: were some of these 53 subjects microfilaraemic? Or were they all amicrofilaraemic? What does "microfilaremia < 1/mm3" mean?

- These 53 patients were all amicrofilaraemic. Given that a microfilaremia was defined as positive in the methodology section when microfilaremia >1/ml, a microfilaremia < 1/ml corresponds to a negative microfilaremia. To clarify this point we have modified in “53 (31.7%) had a negative microfilaremia”.

Comment 22 : Lines 162-163: how many subjects were tested by immunoelectrophoresis?

- 125 IE were performed. We added that information in the revised version.

Comment 23 : Line 165: what does "prior history of loiasis" mean?

- some patients reported previous episodes of loiasis in the past.

Comment 24 : In Table 2, clarify that the mean microfilaraemias are arithmetic means, and that they were calculated on those subjects who were microfilaraemic

- done

Comment 25 : Line 198: how many patients received 1, 2, 3 etc. single doses of ivermectin? What was the interval between the doses?

- The repartition of the number of courses is shown in the table. We added in Table 4 (legend) the percentage for each class.

<table>
<thead>
<tr>
<th>N° of courses of IVT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total : 129</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N° of patients</td>
<td>(113 +26 – 10 IVT+ABZ)</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(62.8%)</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(17.8%)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7.7%)</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.2%)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.3%)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3.1%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- the interval between the doses is not available

Comment 26 : In Table 4, could the number of microfilaraemic and amicrofilaraemic subjects (and the range of microfilarial densities) be given for each drug regimen?

- The database manager and statistician who worked on this study are no longer available and not recoverable. Asking new professionals who are not familiar with the study to look into the database is not easy and will require time (and funding). Therefore, although relevant, it is unfortunately impossible to meet this demand, particularly because of the deadlines for submitting the revised version of the manuscript.

Comment 27 : Line 212: what microfilarial density was reached after filariopheresis, just before starting DEC treatment?

- This data is not available in the database

Comment 28 : Lines 217 and 223-228: the authors suggest that the distribution of loiasis goes further west of the presently-admitted limit of South-East Benin. This is very interesting (and might be
possible) but needs to be much more strongly supported. More information should be presented in the paper on possible travels into endemic areas of those 3 subjects supposed to have been infected in Mali, Ivory Coast or Rwanda (i.e. can such a travel history be definitely excluded?). The clinical and biological features of these cases should also be given. At line 228, the authors mention a paper presenting a trial conducted in Benin; but Klion et al. do not suggest in this paper that "it is likely that a low level of transmission still remains in remote forested areas".

as already stated in the response to the comment 17, the information provided by the clinicians in charge of the patients stipulates that the question was specifically asked to the patients and that no other trip in a more classically endemic area was found. The authors do not pretend to assume the extension of the zone of endemic loiasis. They only observed in the literature that authors stated that the western part of the African rain forest has been considered in the past as a possible endemic zone for loiasis. However, due to this weakness in retrospective studies on the reliability of patient data, it is not impossible that the patients concerned did not mention, for one reason or another, another stay in endemic areas. Therefore, if the hypotheses to explain these atypical contamination areas are to be prioritized, it is reasonable to put this residual transmission hypothesis only in second place. We have clarified the text along these lines.

Furthermore, the authors do not attribute this hypothesis to Klion. The modifications made resolve this ambiguity.

We added in Table 1 main data regarding these 3 patients.

Comment 29: In the discussion, the authors should certainly mention that new tests are being developed to diagnose infection with Loa loa. A rapid antibody-detection test, whose performance in terms of sensitivity and specificity has still to be evaluated, has been developed (Pedram et al., PLoS Negl Trop Dis. 2017 Jul 27;11(7):e0005741). Should the first encouraging results be confirmed, this new tool would certainly be most useful to diagnose loiasis, and particularly occult (=amicrofilaraemic) loiasis.

we strongly agree with the reviewer that current diagnostic tests are underperforming and that new tests are needed. The test referred to by the reviewer seemed to the authors too preliminary in its evaluation to be mentioned. Nevertheless we added a sentence, partly based on the reviewer's commentary, to refer to it as it represents a real hope of improvement.

Reviewer 2:
We agree with the reviewer's general comment, particularly with regard to the standardization of diagnostic and treatment evaluation criteria, but as the reviewer himself says, at the time the study protocol was drafted (1992), during its conduct (1993-2013) (and even now) its criteria did not exist. The very recent diagnostic test to which he refers (Pedram 2017) which seems very specific and will probably be considered as the reference was obviously not available. As for the inclusion criteria that can always be discussed and that necessarily have limits, they result from a deliberate choice and have had the advantage of identifying the frequency of asymptomatic forms.

Comment 1: page 1, line 1-2: loiasis is present only in central and west Africa, so it would be better to classify the patients from endemic or non endemic countries, in the title and in the whole article.

this point refers to the 3 patients who reported having stayed in an area considered non-endemic (Ivory Coast, Mali, Rwanda). All other patients reported having been infected in a Central African endemic country. As mentioned in the discussion and reinforced in the revised version, the retrospective nature of the study does not make it possible to eliminate the possibility of an error or omission (for whatever reason) on the part of these patients in a stay in another (endemic) country than the one declared. Consequently the hypothesis of a persistent transmission at a low level in isolated areas of these formerly endemic areas (according to authors cited in the manuscript) cannot be
presented as the main hypothesis. Moreover, it seems to the authors that these 3 cases are too small in number to justify articulating the article between these 2 situations.

Comment 2: page 2, line 27: if loiasis has become a rare cause, the authors should specify if before it wasn't a rare cause

- the answer to this request is difficult because this reference to the rarity of the diagnosis of loiasis is in the abstract and it is difficult to further develop here this point. The authors propose to change “has become a rare cause…” in “is a rare cause”

Comment 3: page 2 line 28. please specify the areas of tropical Africa

- we replaced “tropical” by “central” Africa

Comment 4: page 2, line 32: in the methods it would be better to specify if the diagnosis of loiasis was made by detection of eyeworm, microfilariae or occult loiasis

- the authors agree that the methodology and in particular the inclusion criteria are not detailed enough in the abstract but this is due to lack of space. As the inclusion in the study was based on a parasitological diagnosis (and not clinical criteria) we added “on the basis of a parasitological diagnosis (microfilariaemia >1/ml and/or serologic tests)”

Comment 5: page 2, line 39-40: it is very strange that the serology resulted positive only in 53% of the patients, while the microfilariaemia was present in the 63% of the patients. it would be logical to expect serology to be positive in cases with positive microfilariaemia and in a large part of those without microfilariaemia

- The authors are not sure they understood this comment correctly. As discussed in the discussion section, it is reported in most studies, consistent with our findings, that a positive serological diagnosis is more frequent in patients of non-African origin (expatriates) while a positive microscopic examination (positive microfilariaemia) is more frequent in African patients. Similarly, studies show that serologies are not always positive when microfilariaemia is present. As in our study there is a predominance of African patients, it is not surprising that positive serologies are less frequent than positive microfilariaemias.

Comment 6: page 2, line 43: it would be better to define the cure rate

- we agree that it is important to give a precise definition of the cure rate but the constraints of space in the abstract do not allow us to do so. It is detailed in the methodology section.

Comment 7: page 3, line 49: please specify the part of Africa (west and central Africa)

- we replaced in the key-words Africa by West and Central Africa

Comment 8: page 3, line 57: it would be better to report the references 6-14 chronologically

- we agree and have changed the order of references

Comment 9: page 4, line 74-76: the meaning of the sentence is not clear

- the first step in patient selection was to have a parasitological diagnosis of loiasis either by microscopy (positive microfilariaemia) or serology (screening and confirmation serologies). Then, for patients diagnosed serologically, considering the limitations of serological tests, only patients with an epidemiological (stay in endemic areas) and/or a clinical history compatible with a loiasis were definitively included. We modified the sentence in the revised version to clarify this point.

Comment 10: page 4, line 76-78: the authors state that they did not include patients who did not fit with epidemiological presentation, but they included patients from Ivory Coast, Mali and Rwanda, countries where Chryrops are not present (countries not considered endemic for loiasis). It would be very interesting to know if these patients were diagnosed with serology or with positive microfilariaemia.

- In accordance with our inclusion criteria, these 3 patients were included despite having declared an area of contamination considered non-endemic because in 2 cases the diagnosis was confirmed by the presence of microfilaria (South Mali and Ivory Coast) and in the 3rd case, where the diagnosis was made by serology, the symptomatology was very suggestive of loiasis (subcutaneous oedema of the ankle). As also requested by the other reviewer, the main data from these 3 patients have been added to Table 1.
As announced in the methodology we wanted to compare African patients and non-African patients. Among the African patients we distinguished 2 groups: VFR patients (patients born in an African country, living in France and returned to their country of origin for the holidays) and African living in Africa but passing through France or recently arrived in France as immigrants.

Strictly speaking, Calabar oedema meets a precise definition detailed in the text. Therefore, other forms of subcutaneous oedema that do not strictly meet this definition (e.g., persistent for more than a week or not located at the extremities) have been classified separately.

As mentioned at the beginning of the comments, it is true that in the absence of a “gold standard” serologic technique, most of the techniques used (apart from ELISA serologies) were not validated commercial kits. However, the diagnostic strategy used in all centres, including 1 or 2 screening techniques and 1 or 2 confirmation techniques with better specificity, increases the probability that they are "true" loiasis diagnoses. However, this is a weakness of this diagnostic mode, but it was everywhere at that time and therefore in the other studies conducted at that time. This is also the reason why we excluded patients diagnosed by serology for whom the area of contamination and/or symptoms were not compatible with a loiasis. In the end, we believe that the probability that fake loiasis cases have been included is not zero but is very low.

The authors are not sure they understood this comment correctly since these results are given page 9/line 184-190 (first draft)

We agree with this comment. However, in situations where parasite eradication is not the objective (i.e. patient usually residing in the endemic area and returning to it) or in situations where the patient has not returned to the practitioner for the treatment by DEC, ivermectin has appeared in the database as the single drug. This is one of the limitations of retrospective studies where the treatment regimen cannot be controlled.

We agree that it would have been interesting to have a standardized follow-up to better evaluate the evolution under treatment. However, as this is a retrospective study, we do not have this opportunity: the follow-up conditions were either not specified in the patient file or, when specified, too variable to be able to analyze the follow-up conditions. For the same reasons it was difficult to define more precise criteria for cure or failure. However, according to the proposals of the other reviewer, we have made some clarifications in the revised version.

We agree that having this data for only 9 patients is frustrating and that the median incubation time calculated on the 9 patients in whom it was possible to do so cannot be extrapolated to all patients in the study. However, this data is rarely available (found in a single study) because it is rare to have patients in a travel configuration where it is possible to calculate it. The authors believe that being able
to indicate to non-specialist in tropical medicine practitioners that symptoms of loiasis can appear long time (up to 18 months in the study) after return is useful information and would prefer to keep this information in the final text. However in the discussion the text has been modified to limit the information to a possible late emergence.

Comment 19 : page 6, table 1: please correct ] instead [ 
Done in the revised version

Comment 20 : page 7, line 152: pruritus is present in patients with loiasis, but it is also present in other filariasis (such as Mansonella perstans infection), so we can't consider it as a specific symptom
We fully agree with this remark. Nevertheless, the practitioners in charge of these patients, in this context of return from an endemic area, considered that this symptom, even if not specific, justified the prescription of a loiasis diagnosis.

Comment 21 : page 8, line 162-163: the meaning of the sentence is not well understood
Among the 92 patients with positive serology, 54 had a specific Loa loa arc at the immunoelectrophoresis, which was a very strong argument for diagnosis.

Comment 22 : page 9, line 184-187: this sentence is very confusing and refers to non-validated serology
Insofar as these serologies concerned cases with a definite diagnosis since the microfilaremia was positive, we also evaluated the sensitivity in these situations of undetermined serologies (positive reaction but below the positivity threshold set by the laboratory). However, the authors agree that this result may be confusing since according to our methodology, serologies with an undetermined result were considered negative. Therefore, in the revised version, we deleted the sensitivity assessment when serology was undetermined.

Comment 23 : page 10, line 191: the authors state that the outcome was present in 165/167 patients, but in the table 4 they reported 44 patients treated with ivermectin lost to follow up. Please specify this discrepancy
we have included in the notion of “outcome” all the situations where we have information about the patients after the diagnosis has been made, including when the patient has been lost to follow-up after treatment has been given. That is why we talk about “outcome” and not “outcome under treatment”. For 2 patients we have no information after diagnosis.

Comment 24 : page 10, line 197-203: considering that in the methods cure and failure definitions are not clear, consequently the outcome is debatable
We understand the reviewer's position because having a very precise definition of the evolution under treatment, as can be done in a prospective study, is always better. However, and as stated in comment 17, it is difficult in a retrospective study to have this precision because the available data generally do not allow it. The authors consider that in this context, the definitions given in the methodology are sufficiently precise to be able to analyze the evolution under treatment.

Comment 25 : page 10, line 195: it would be better to specify how many patients were treated with 1, 2, 3, 4, 5 or 6 courses of ivermectin
We agree and added these data in table 4.

Comment 26 : page 11, line 207: the authors report that 4 out of 10 people treated with one course of ivermectin received also albendazole. Then the patients were not treated with ivermectin alone. Moreover, albendazole has a macrofilaricidal effect (Klion AD, Horton J, Nutman TB. Albendazole therapy for loiasis refractory to diethylcarbamazine treatment. Clin Infect Dis. 1999 Sep;29(3):680-2.) so the cure presumably resulted from the combination of the 2 drugs
We totally agree with this comment. The choice to include these 4 patients in the "ivermectin" group results from the fact that creating an additional group with only 4 patients made no clinical sense. We believe that these 4 cases did not significantly influence the evaluation of the efficacy of this group and that readers will be able to integrate the fact that albendazole played a role in the treatment course.

Comment 27 : page 11, line 218: without specific criteria to define cure and failure, this manuscript
cannot contribute to assess the response to treatment

We are well aware of the limitations of this study that are consistent with those of retrospective studies in general and have clearly indicated this in the manuscript. To reinforce these limitations, the authors added at the end of this sentence “… although the limitations of a retrospective study should lead to caution in interpreting these results”. Furthermore the authors also believe that the definitions of full cure, partial cure and failure provided in the methodology section, even if not perfect, are clinically and parasitologically relevant. Since the rarity of imported loiasis makes a randomized prospective study practically impossible, we believe that these data based on a significant number of patients can provide guidance in the absence of a validated strategy in this particular context of imported pathology. In addition, our suggestion to prioritize DEC, possibly preceded by ivermectin, particularly when the parasitic load is high, is in line with the therapeutic regimens proposed in the literature.

Comment 28: if the patients coming from non endemic countries (Mali, Cote d'Ivoire, Rwanda) were real cases of loiasis, this discovery would deserve a publication in itself, but to do so the authors should demonstrate the presence of the adult worm or microfilariae and be 100% sure that these patients never stayed in endemic countries

the authors share the reviewer's interest in this plausible hypothesis because these countries are former endemic areas and in 2 cases (South Mali and Ivory Coast) the diagnosis is certain with the presence of microfilariae. For the patient supposed to be infected in Rwanda, there is little doubt about the diagnosis because it was made by a positive serology with a specific arc. Unfortunately, the retrospective nature of the study, with the impossibility of re-questioning patients (even though the doctors in charge of these patients specifically stated that there had been no other countries visited), does not guarantee 100% that these patients have not also travelled to an endemic area. For this reason, we have amended the text in the revised version to indicate that this "residual" transmission hypothesis cannot be the priority hypothesis.

Comment 29: the authors cannot demonstrate a mean incubation time, based on data from 9 patients

We agree. Please refer to reply to comment 18.

Comment 30: the other symptoms may be due to co-infections

In the database the number of infections or diseases associated with loiasis was very limited and none could explain these symptoms.

Comment 31: it is very difficult to differentiate between Calabar swelling and migratory oedemas.

The choice to differentiate these 2 types of subcutaneous oedemas associated with loiasis can always be discussed, but this is the one that was done in the study methodology. Applying the definition provided, the physicians participating in the study seem not to have had any difficulty in classifying these 2 types of oedema.

Comment 32: the patients described by the authors have a mean microfilaremia of 2586 and 1247, while the severe adverse effects of Dec and ivermectin were reported in patients with higher microfilaremia

The authors are not sure they understood this comment correctly since we did not observe any severe adverse effect in our study.