OPEN PEER REVIEW

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Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
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- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jundishapur Journal of Microbiology
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Peer Review Report for "Molecular Characterization of Visceral Leishmaniasis in Asymptomatic Dogs in North Khorasan, Northeastern Iran"

Author(s): Roozbeh Taslimian, Bahar Shemshadi, Adel Spotin, Reza Fotouhi Ardakani, Parviz Parvizi

Review Timeline:

Submit Date: 10 Dec 2019
Revised Date: 19 Jan 2020
Accepted Date: 23 Jan 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0)

31 Dec 2019

Dear AE,
I am pleased to have the opportunity to revise the present manuscript (ID: 100078). The title is fascinating. The manuscript needs some revision that are listed below. It is strongly recommended to benefit an English editor to proofread the manuscript.
Abstract:
- “In Iran” in the first sentence “In Iran, Leishmania infantum parasites are principal causative…” should be at the end.
- Please give accurate details about sampling. “Sampling was performed from blood, snout, and conjunctiva” is not clear.
- Please change the conclusion. However the title is about the molecular diagnosis of Leishmania, it was concluded that the sampling method is not invasive!
Introduction:
- Canis familiaris should be Italic.
Material and Methods:
- Please add the Ethical code of the project.
- Please add the full sequences of primers.
Discussion:

- The first letter (N) as well as (P) in North Khorasan Province should be the capital.
- It is not necessary to report detailed statistics from Sheikh Hospital.
- This sentence is not clear. (If the test result is positive, the infected animal can be treated or preventive measures could be taken). Furthermore, the mentioned sentence doesn't need any references. References are associated with previous sentences. Please correct that.
- Please consider changing this part "By employing the sensitive and specific ITS-rDNA gene (35), approximately 30% of sampled dogs were found to be infected by L. infantum (n=10) and L. tropica (n=1), the most important, recognized causative agent of VL in dogs (23, 42, 43)." Leishmania tropica is not the most causative agent of VL in dogs!
- Please mention the studies in detail about infected cases conjunctival samples (Our findings were also been confirmed by previous studies (30, 44).)
- Please change "spot-on product", it is not an appropriate phrase. (Prevention of infection can be achieved by spot-on products)
- It is recommended to focus on molecular findings rather than the sampling method in conclusion.
- Discussion does not follow a steady trend. It is kind of confusing. Referring is not properly done. Molecular results have not been compared with other study results.

Thanks.

REFEREE: Reviewer | **Revision** (0) 31 Dec 2019

Dear AE,

The manuscript entitled" Molecular characterization of visceral leishmaniasis within different organs...." needs major revisions.

It must be completely revised. It not well written. The writing language of the article is very weak.

1. Title

Title should be precise and clear.

Molecular characterization of visceral leishmaniasis? or Molecular characterization of leishmania infantum?

"north Khorasan" should be changed to "North Khorasan".

What is the mean of different organs?

Which group of dogs have been studied? Domestic, stray, or both of them?

2. Abstract

Background:

the authors have written that " In Iran, Leishmania infantum parasites are principal causative agents of visceral leishmaniasis (VL) which threats a wide range of humans and canines. "

#. Principal or main? Threats or threat? Canines or canids?

..... a wide range of humans? Do the authors think that visceral leishmaniasis is a common disease in human populations in Iran?

3. Objectives: the authors have said that "Our aim was surveying Leishmania parasites species…"

I did not understand. Which species of Leishmania? Do they were seeking for all Leishmania species or their aim was only Leishmania infantum?

#..... in endemic areas..... what do they mean?

during July and August 2018: Do you mean July to August 2018?
#. “DNA of collected samples was extracted, amplified, and sequenced by targeting of ITS-rRNA gene. “ This sentence means that they extracted DNA using ITS gene.
Why they did not use visceral organs?
4. Results: A total of 118 samples were obtained from 37….
The authors must be writing the sample numbers according to the number of studied dogs (37).
Thanks.

Bahador Shahriari: Reviewer | **Revision (0)** 1 Jan 2020

Dear AE,
The current study describes the prevalence of Leishmania in dogs in North Khorasan, northeastern Iran. As data about canine leishmaniasis are lacking in this area, the study has got enough merits to be considered for publication. However, these are few concerns regarding the methodology, and findings of the study which need to be considered by the authors and provide appropriate details.
1. Title: Samples from blood, snout, and conjunctiva have been tested which does not imply many different organs. So the title should be changed to: "Molecular characterization of visceral leishmaniasis in asymptomatic dogs in north Khorasan, northeastern Iran"
2. Abstract
3. In the Abstract section of the article, the target gene is mentioned as ITS-rRNA while in the title and keywords it is cited as ITS-rDNA.
4. The sentence "The highest number of infections was in the age group of 5-10 years old of dogs indicating this group is more sensitive to VL in this region is related to the Results, not Conclusion.
5. The conclusion is not based on the findings of the study. Neither bone marrow nor lymph node samples have been evaluated in this study. Therefore the related sentence should be modified.
Introduction
6. Canis familiaris should be italic.
7. Please rewrite this sentence "The objective of this study was performed to detect causative agents of VL in asymptomatic"
8. Study area: what the authors mean by CVL patients in the study area? Canine Visceral Leishmaniasis (CVL)!!
9. Sample collection. Samples have been collected from blood, snout, and conjunctiva which are three different samples. What the authors mean by "four different samples" here.
10. Please provide the reference number for the ethical approval of the study.
11. Which part of the blood samples has been used for DNA extraction, whole blood or buffy coat?
12. Please provide brief details about the PCR protocol.
13. Please prove information about the positive control for L. tropica and L. infantum.
Results
14. Do any of the sequences deposited in the GenBank?. If it is so, please provide the accession numbers.
15. How many samples have been sequenced? Were there any inter-and intra specific

variations of Leishmania isolated from the dogs? Please explain. Providing a multi-alignment figure of sequences would help in this case.
16. Please provide a figure of agarose gel which show the PCR products of the isolates and also the positive control for L. tropica and L. infantum
Discussion
17. Change cattle to cattle.
18. In paragraph 3, the authors are talking about 15 reported CVL samples during 2006-2017. Are they human cases or canine visceral leishmaniasis (CVL). If they are human cases, why referred to as CVL.
19. Authors are saying that: conjunctiva, oral mucosa or snout and blood are both reliable and suitable for molecular studies" but the finding of the study does not support this concept.
20. Please change MOST to most.
21. Please provide a concise and informative conclusion, based on the findings of the study, at the end of the Discussion section.
22. Please provide a number for figures.
23. Data presented in the second figure are already available in the text. This figure should be omitted.
24. In the third figure, only two out of 10 isolated samples in this study are included in the phylogenic tree. The authors are requested to include all the 10 isolated samples in the tree (even if they are 100% similar).
25. There are so many typo and syntax errors in the manuscript which need to be rigorously revised.
Thanks.

REFEREE: Associate Editor | **Revision** (0) 14 Jan 2020

Dear EIC,
The manuscript needs several correction as reviewers comments.
Thanks.

REFEREE: EIC | **Revision** (0) 18 Jan 2020

Dear Authors,
Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below. The reviewers recommend reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments. Please answer point by point to the reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers' queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding.
When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed. In addition, JJM INSTRUCTION attached to mail, Pleases find it.
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Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Ref.: Ms. No. 100078

Molecular characterization of visceral leishmaniasis within different organs of asymptomatic dogs by targeting ITS-rDNA gene in north Khorasan, northeastern Iran

Jundishapur Journal of Microbiology 19th January 2020

Dear Prof. Ali Zarei Mahmoudabadi ;

Editor in chief of ***Jundishapur Journal of Microbiology***

Thanks to my MS reviewers for their comments and advices.

This is my point-by-point response sheet to all issues raised by the referees: In texts of MS, comments and changes of each reviewer marked by different color.

Reviewer 1: Green

Reviewer 2: Yellow

Reviewer 3: Blue

Reviewer 1:

The current study describes the prevalence of Leishmania in dogs in North Khorasan, northeastern Iran. As data about canine leishmaniasis are lacking in this area, the study has got enough merits to be considered for publication. However, these are few concerns regarding the methodology, and findings of the study which need to be considered by the authors and provide appropriate details.

1. Title: Samples from blood, snout, and conjunctiva have been tested which does not imply many different organs. So the title should be changed to: "Molecular characterization of visceral leishmaniasis in asymptomatic dogs in north Khorasan, northeastern Iran"

Done and highlighted

2. Abstract

3. In the Abstract section of the article, the target gene is mentioned as ITS-rRNA while in the title and keywords it is cited as ITS-rDNA.

Done and highlighted

4. The sentence "The highest number of infections was in the age group of 5-10 years old of dogs indicating this group is more sensitive to VL in this region' is related to the Results, not Conclusion.

Done and highlighted

5. The conclusion is not based on the findings of the study. Neither bone marrow nor lymph node samples have been evaluated in this study. Therefore the related sentence should be modified.

Done and highlighted

Introduction

6. Canis familiaris should be italic.

Done and highlighted

7. Please rewrite this sentence "The objective of this study was performed to detect causative agents of VL in asymptomatic"

Done and highlighted

8. Study area: what the authors mean by CVL patients in the study area? Canine Visceral Leishmaniasis (CVL)!!

Done and highlighted

9. Sample collection. Samples have been collected from blood, snout, and conjunctiva which are three different samples. What the authors mean by "four different samples" here.

The authors wanted to express both right and left conjunctiva were sampled. Therefore were mentioned four samples. To satisfy the reviewer’s opinion we changed the statement to “three different samples”.

10. Please provide the reference number for the ethical approval of the study.

IR.PII.REC.1395.20. Done and highlighted

11. Which part of the blood samples has been used for DNA extraction, whole blood or buffy coat?

Whole blood. Done and highlighted

12. Please provide brief details about the PCR protocol.

The amplification reaction was carried out in a total of 20µl containing 1× Taq polymerase buffer, 1.5 mM MgCl2, 60µM of each dNTP, 1 unit Taq polymerase (pre-prepared 2× Amplicon master mix), 1µM primer ITS1F, 1µM primer ITS2R4 and 1.5µl (5–10 ng/µl) of DNA extracted from samples. The mixture was incubated in a mastercycler gradient Eppendorf Thermocycler (0.2 ml block) at 94 ◦C for 5 min followed by 37 cycles, each consisting of 45 s at 94 ◦C, 45 s at 60 ◦C and 30 s at 72 ◦C. After the last cycle, the extension was continued for a further 10 min then held at 4 ◦C (Parvizi et al 2005 with minor modification).

Done and highlighted

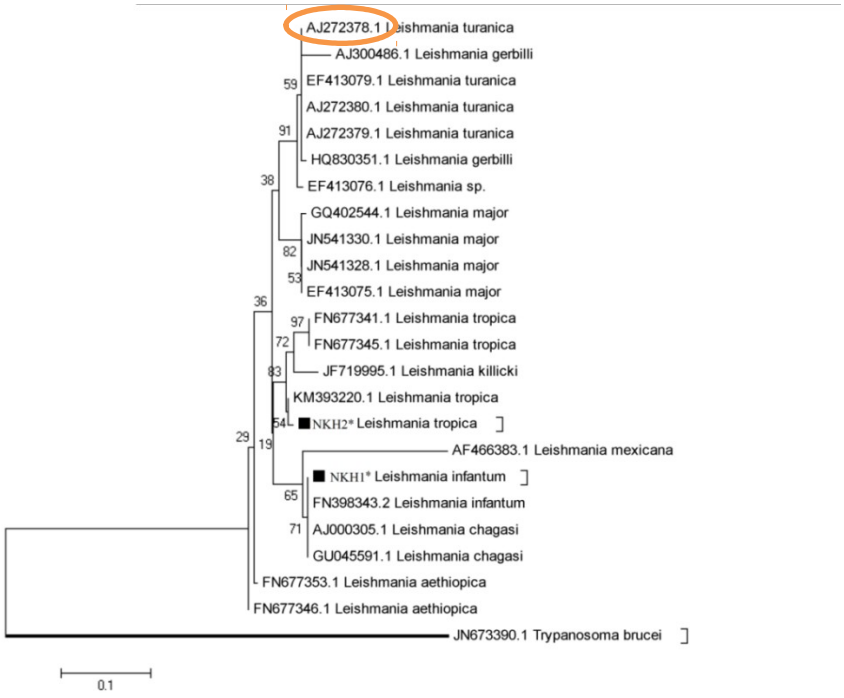
13. Please prove information about the positive control for L. tropica and L. infantum.

L. infantum (Lon 49) and *L. tropica* (MHOM / IR / 02 / Mash10). Done and highlighted

Results

14. Do any of the sequences deposited in the GenBank?. If it is so, please provide the accession numbers.

The accession numbers of each sample used to draw the phylogenic tree is brought in fig3.



All *Leishmania* positive samples (480 bp) amplified and showed DNA band on agarose gel. All 11 samples of infected dogs were sent for sequencing after editing and alignments a phylogenic tree was constructed using MEGA7 software. Sequences submitted GenBank including

"KM393220.1 for *L. tropica*", "FN398343.2 as *L.infantum*", "EF413075.1 as *L.major*" and for out-group *Trypanosoma brucei* with access number of "JN673390.1" were employed.

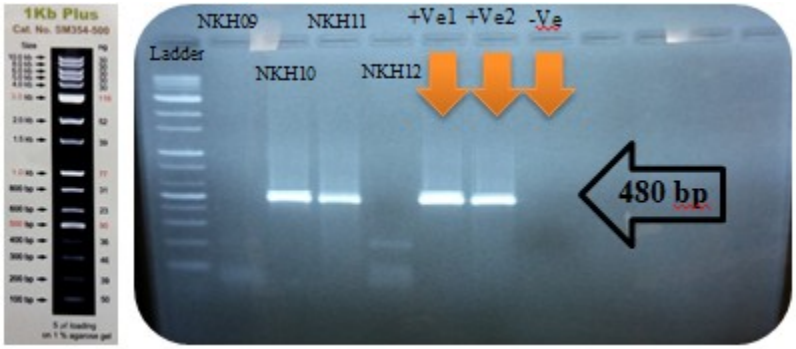
Done and highlighted

15. How many samples have been sequenced? Where there any inter- and intra specific variations of Leishmania isolated from the dogs? Please explain. Providing a multi-alignment figure of sequences would help in this case.

11 samples of infected dogs were sent for sequencing.

No inter- and intra-specific variations were seen.

16. Please provide a figure of agarose gel which show the PCR products of the isolates and also the positive control for *L. tropica* and *L. infantum*



.Gel electrophoresis for *L. infantum*(+Ve1), *L. tropica*(+Ve2), negative and four samples

Done and highlighted

Discussion

17. Change cattel to cattle.

Done and highlighted

18. In paragraph 3, the authors are talking about 15 reported CVL samples during 2006-2017. Are they human cases or canine visceral leishmaniasis (CVL). If they are human cases, why referred to as CVL.

Done and highlighted

19. Authors are saying that: conjunctiva, oral mucosa or snout and blood are both reliable and suitable for molecular studies" but the finding of the study does not support this concept.

Referring on the results and data in table 1, *Leishmania* infection was detected from blood, snout and conjunctiva samples of asymptomatic dogs. Using this noninvasive method, the canines were kept alive which is useful in treatment and follow up programs. Also the sampled dogs are

sheepdogs and are valuable for the villagers of endemic regions; sampling of spline and lymph nodes would kill the dog and harm the dogs owner.

20. Please change MOST to most.

Done and highlighted

21. Please provide a concise and informative conclusion, based on the findings of the study, at the end of the Discussion section.

Done and highlighted

22. Please provide a number for figures.

Done

23. Data presented in the second figure are already available in the text. This figure should be omitted.

Done

24. In the third figure, only two out of 10 isolated samples in this study are included in the phylogenic tree. The authors are requested to include all the 10 isolated samples in the tree (even if they are 100% similar).

“Including all the 10 isolated samples in the tree (even if they are 100% similar)” in phylogenetic tree is wrong. Only sequences can include in phylogenetic analyses which at least has one nucleotide different. I refer Reviewer to REF No 35 and 36 of this MS.

25. There are so many typo and syntax errors in the manuscript which needs to be rigorously revised.

Done and thank you.

Reviewer 2:

Dear associate editor

I am pleased to give the opportunity to revise the present manuscript (ID: 100078). The title is fascinating. The manuscript needs some revision that are listed below. It is strongly recommended to benefit an English editor to proofread the manuscript.

Yours,

Abstract:

- “In Iran” in the first sentence “In Iran, Leishmania infantum parasites are principal causative…” should be at the end.

Done and highlighted

- Please give accurate details about sampling. “Sampling was performed from blood, snout and conjunctiva” is not clear.

Done and highlighted

- Please change the conclusion. However the title is about the molecular diagnosis of Leishmania, it was concluded that the sampling method is not invasive!

Done and highlighted

Introduction:

- Canis familiaris should be Italic.

Done and highlighted

Material and Methods:

- Please add Ethical code of the project.

IR.PII.REC.1395.20 Done and highlighted

- Please add the full sequences of primers.

Done and highlighted

Discussion:

- The first letter (N) as well as (P) in Nnorth Khorasan Province should be capital.

Done and highlighted

- It is not necessary to report about detailed statistic from Sheikh Hospital.

I respect Reviewer 2 advice but Reviewer 1 advised to keep and revise. Done and highlighted.

- This sentence is not clear. (If the test result is positive, the infected animal can be treated or preventive measures could be taken). Furthermore the mentioned sentence doesn’t need any references. References are associated to previous sentences. Please correct that.

Done and highlighted

- Please consider changing this part “By employing the sensitive and specific ITS-rDNA gene (35), approximately 30% of sampled dogs were found to be infected by L. infantum (n=10) and L. tropica (n=1), the most important, recognized causative agent of VL in dogs (23, 42, 43).” Leishmania tropica is not the most causative agent of VL in dogs!

Done and highlighted

- Please mention the studies in details about infected cases conjunctival samples (Our findings were also been confirmed by previous studies (30, 44).)

Done and highlighted

- Please change “spot on product”, it is not appropriate phrase. (Prevention of infection can be achieved by spot on products)

Done and highlighted

- It is recommended to focus on molecular findings rather than sampling method in conclusion.

Done and highlighted

- Discussion does not follow a steady trend. It is a kind of confusing. Referring is not properly done. Molecular results have not been compared with other study results.

Thank you. Revised and molecular results compared with other study results and added in Discussion

Reviewer 3:

The manuscript entitled" Molecular characterization of visceral leishmaniasis within different organs...." needs major revisions.

It must be completely revised. It not well written. The writing language of the article is very weak.

1. Title

Title should be precise and clear.

Molecular characterization of visceral leishmaniasis? or Molecular characterization of leishmania infantum?

Regarding Reviewer 1 advice, Title was corrected. Done and highlighted

“north Khorasan” should be changed to “North Khorasan”.

Done and highlighted

What is the mean of different organs?

Removed and changed

Which group of dogs have been studied? Domestic, stray, or both of them?

Neither, sheepdogs

2. Abstract

Background:

the authors have written that " In Iran, Leishmania infantum parasites are principal causative agents of visceral leishmaniasis (VL) which threats a wide range of humans and canines. "

#. Principal or main? Threats or threat? Canines or canids?

Main, threat and canines

Was changed and highlighted

…….. a wide range of humans? Do the authors think that visceral leishmaniasis is a common disease in human populations in Iran?

VL is endemic in North East and North West, also Shiraz and Kerman Provinces of Iran.

3. Objectives: the authors have said that "Our aim was surveying Leishmania parasites species…"

I did not understand. Which species of Leishmania? Do they were seeking for all Leishmania species or their aim was only Leishmania infantum?

All *leishmania* species

#….. in endemic areas….. what do they mean?

The areas in which the disease is seen and spread annually

During July and August 2018: Do you mean July to August 2018?

Yes. 28th July till 4th August 2018

Done and highlighted

#. "DNA of collected samples was extracted, amplified and sequenced by targeting of ITS-rRNA gene. " This sentence means that they extracted DNA using ITS gene.

Done and highlighted

Why they did not use visceral organs?

The authors wanted the sampling to be non-invasive.

4. Results: Total of 118 samples were obtained from 37….

The authors must be writing the sample numbers according to the number of studied dogs (37).

Done and highlighted

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 23 Jan 2020

Dear Authors,
According to the associated editor report, your manuscript is accepted for publishing in JJM and send to the publisher for the next process.
Note:
Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. http://single.demo.pub/en/knowledgebase/category/tree.html#orcid.html
Thanks.