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PROCEEDINGS B

Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease

Carezza Botto-Mahan, Antonella Bacigalupo, Juana P. Correa, Francisco E. Fontúrbel, Pedro E. Cattan and Aldo Solari

Article citation details
Proc. R. Soc. B **287**: 20193018.
http://dx.doi.org/10.1098/rspb.2019.3018

Review timeline

Original submission:	16 July 2019
1st revised submission:	31 December 2019
2nd revised submission:	12 February 2020
Final acceptance:	13 February 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-1670.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is a well conducted and interesting paper that aim understanding the variables that modulate the transmission of parasites by vectors. The host parasite system studied herein (T. cruzi - mammal host and triatomine) authors who are highly experienced in the topic , propose to take into account the ecological characteristics (focus on life history and behavior) of the vector species under study, which in their case is triatomine. Mepraia spinolai . Authors discuss transmission being dependent on both, vector and mammalian density and in this sense the model studied is the transmission of T. cruzi by a vector species that does not perform active search and does not move as much as is the case. of M. spinolai.

Actually, this is a manuscript that should be accepted for publication but some small aspects need to be clarified:

1) I suggest to the authors that they prefer the term parasite rather than pathogen since pathogenicity is a trait that in not strictly dependent on host instead, it depends on many variables.

2) Did the authors calculate the area in which the insects were scattered? The number of insects caught by me does not seem to be enough to define density.

3) T. cruzi transmission occurs in almost all diverse environments extending from Southern North America to Southern South America. The area focused in this study has many ecological peculiarities, among which, a relatively poor mammal fauna, only three dominant species and one single vector species that does not make large displacements. Two points: How far can authors generalize or what metrics they propose to be used to compare findings across different environments?

4) What is the evolutionary stage of insects? In other words, what was the population structure of the colonies like? Would this not be a variable to evaluate as well? First stage nymphs for example move less than more mature nymphs

5) How to define the variables to carry out comparative studies?

6) Preference of triatomines by infected mammals is a trait that should be considered with extreme caution.

7) As far as I could see, the oral route was not included among the variables. This is a point that deserves at least to be discussed. At least in laboratory conditions, rodents may prey on triatomines or become infected during grooming their fur eventually contaminated by infected triatomine feces.

8) Finally, it must be considered that the different T. cruzi DTUs establish different patterns of infection in the different animal species. How could this aspect interfere with authors observations ?

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Acceptable

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author

This manuscript is about the relationship between host diversity (and identity) on the transmission of Trypanosoma cruzi, a protozoan parasite that is the etiological agent of Chagas disease. The effects of hosts were tested against three response variables that came from the only parasite vector in the area, the wingless triatomine bug Mepraia spinolai. The authors wanted to know which of three infection parameters in vectors were more related to host variables. In general, this topic could be of interest of the journal audience. There is a high interest in how is the intensity and direction of the relationship (if any) between biodiversity and disease risk (i.e. vector infection prevalence or density), however, there are very few vector-borne diseases in which this relationship has been studied. As an example, Lyme disease, in temperate ecosystems is one of the most studied systems, but tropical and subtropical zoonosis remain to be incorporated in a global discussion about how biodiversity can protect people's health. In this sense, I celebrate the attention paid by the authors on Trypanosoma cruzi transmission since Chagas disease is one of the most important neglected diseases in America. However, I have some concerns about the way the authors described their methods and results, mainly regarding the accuracy and precision of their estimations of hosts and vector density. Overall, they calculate density as a simple number of individuals over sampling effort (sampling area for the case of rodents and colony size? in the case of vectors), however, this assumes that for rodent there are no differences in capture probability (for instance, capture-recapture rates) related with behavioral responses toward traps (some rodents are trap lovers while other species are trap haters, etc), between species or that for bugs there are no biases in collection success. Therefore, I recommend estimating density from models that can incorporate assumptions related to the sampling design. The authors made a very good job collecting a high number of samples in both, vectors and rodents, so I am confident that they can estimate realistic densities.

Methods:

The authors provide very little information about how rodent density was estimated. This is crucial to determine a confident magnitude for predictors. The trapping design is a line-transect or a grid? They set up two lines (transects) in parallel separated by 10m (100 traps) so this can constitute a grid. What model was used to estimate the density? His was estimated for each species (infected or total) per species/set or over the three sets taken as a whole?

Line 198: vector infection probability: the infection status is infected or not infected, therefore, their probability according to the definition of authors will be 1 or 0 per specimen, is that right? How the authors can calculate confidence intervals based on this procedure? The mean of these values over the sampled number is not that the prevalence?

Results:

Line 246-247 These numbers are intervals of confidence at what level? Or is a range considering all the sampling years? (Also this comment applies to Line 226)

Discussion:

Line 268: What authors mean by depauperate? Is this rodent community affected by a disturb (chronic?) that is causing a habitat filtering? Or authors refer to a community with a natural and stable low diversity caused by biogeographic processes?

Line 271-272: What the authors mean for a highly complex dynamics of parasite transmission? It seems that it is dependent on the rodent density which suggests a density-dependent process led by hosts, however, authors did not include vector abundance (or density) which could also have an effect on vector infection if host availability can influence vector competition for blood meals. Additionally, authors did not evaluate which factors could cause the interannual variations on rodents and vectors (temperature, humidity, resources, etc?)

Line 277-279: The diversity gradient on this study area is maybe too narrow to observe such a diversity effect if relevant, however, most important in this kind of community, is if the species identity is equally relevant for a vector blood meal and parasite transmission? There were vector colonies associated with different species dominance that also differed in transmission dynamics?

Line 290-291: This pattern was also reported by Ramsey et al. 2009 Plos One (https://doi.org/10.1371/journal.pone.0046013) in Mexico but the absence of a statistical difference between adult and juvenile T. cruzi prevalence did not support the juvenile dilution hypothesis.

Line 295-296: How the authors statistically compared the three models?

Decision letter (RSPB-2019-1670.R0)

22-Oct-2019

Dear Dr Botto:

I am writing to inform you that your manuscript RSPB-2019-1670 entitled "Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.

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Sincerely,
Dr Daniel Costa
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Thank you for submitting your manuscript “Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease” To Proceedings B. I have now received two reviews and evaluated the manuscript myself. While we all find the work interesting and the manuscript generally well-written, several important issues have been raised by the reviewers. In particular, reviewer 2 makes an important point about how behavioral differences are likely to influence small mammal and vector trapping success. These could undermine the validity of the conclusions and need to be addressed through more rigorous abundance modeling approaches. Several areas for additional discussion are also noted by the reviewers. For example, reviewer 1 highlights the uniqueness of the study setting and questions how representative it is of other areas.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
This is a well conducted and interesting paper that aim understanding the variables that modulate the transmission of parasites by vectors. The host parasite system studied herein (T. cruzi - mammal host and triatomine) authors who are highly experienced in the topic , propose to take into account the ecological characteristics (focus on life history and behavior) of the vector species under study, which in their case is triatomine. Mepraia spinolai . Authors discuss transmission being dependent on both, vector and mammalian density and in this sense the model studied is the transmission of T. cruzi by a vector species that does not perform active search and does not move as much as is the case. of M. spinolai.

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Referee: 2

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Line 268: What authors mean by depauperate? Is this rodent community affected by a disturb (chronic?) that is causing a habitat filtering? Or authors refer to a community with a natural and stable low diversity caused by biogeographic processes?

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Line 295-296: How the authors statistically compared the three models?

Author's Response to Decision Letter for (RSPB-2019-1670.R0)

See Appendix A.

RSPB-2019-3018.R0

Review form: Reviewer 2

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
I appreciate the attention that the authors paid to my comments/suggestions. I agree with the changes made in this new manuscript and have not further questions. I recommend this paper for publication in this Journal.

Decision letter (RSPB-2019-3018.R0)

11-Feb-2020

Dear Dr Botto

I am pleased to inform you that your manuscript RSPB-2019-3018 entitled "Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into https://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes

you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
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If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Daniel Costa
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).
I appreciate the attention that the authors paid to my comments/suggestions. I agree with the changes made in this new manuscript and have not further questions. I recommend this paper for publication in this Journal.

Author's Response to Decision Letter for (RSPB-2019-3018.R0)

See Appendix B.

Decision letter (RSPB-2019-3018.R1)

13-Feb-2020

Dear Dr Botto

I am pleased to inform you that your manuscript entitled "Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

ANSWER TO REVIEWERS' COMMENTS

MS RSPB-2019-1670

Associate Editor
Board Member 1

1. While we all find the work interesting and the manuscript generally well-written, several important issues have been raised by the reviewers. In particular, reviewer 2 makes an important point about how behavioral differences are likely to influence small mammal and vector trapping success. These could undermine the validity of the conclusions and need to be addressed through more rigorous abundance modeling approaches. Several areas for additional discussion are also noted by the reviewers. For example, reviewer 1 highlights the uniqueness of the study setting and questions how representative it is of other areas.

A: Please find below our answers to the issues raised by both reviewers, including the points you mention. Our answers are marked with an "A:".

Reviewer(s)' comments to author

Referee 1

1. This is a well conducted and interesting paper that aim understanding the variables that modulate the transmission of parasites by vectors. The host parasite system studied herein (T. cruzi - mammal host and triatomine) authors who are highly experienced in the topic, propose to take into account the ecological characteristics (focus on life history and behavior) of the vector species under study, which in their case is triatomine, Mepraia spinolai. Authors discuss transmission being dependent on both, vector and mammalian density and in this sense the model studied is the transmission of T. cruzi by a vector species that does not perform active search and does not move as much as is the case. of M. spinolai.

A: We appreciate your comments. We provide detailed responses to each comment below.

Actually,this is a manuscript that should be accepted for publication but some small aspects need to be clarified:

1) I suggest to the authors that they prefer the term parasite rather than pathogen since pathogenicity is a trait that is not strictly dependent on host instead, it depends on many variables.

A: We have changed the word "pathogen" by "parasite" throughout the manuscript, as suggested by Reviewer 1. Please see lines 52 and 65.

2) Did the authors calculate the area in which the insects were scattered? The number of insects caught by me does not seem to be enough to define density.

A: We appreciate the reviewer's comment on this issue. We used previously gathered data regarding home range/maximum dispersal distance that was measured in our study site during the austral summer season (please see Botto-Mahan *et al*. 2005, doi: 10.1016/j.actatropica. 2005.05.001). This action ratio was assigned to each colony where we captured insects. We are aware that there could be variability in the dispersal distance/area in which the insects were scattered. However, given that these insects locate underneath rocks until they detect a prey (i.e., sit-and-wait strategy), we are unable to know the exact point of origin of their dispersal, nor calculate the under-rock distances they travelled until reaching the person doing the sampling (i.e., potential prey). Notwithstanding, insects from all colonies were captured using the same collecting procedure, by the same researcher and only on sunny days, within the period of maximum activity for this triatomine species, and with the same sampling effort. Therefore, we think our proxy of density measure is comparable among colonies. We clarified this point in the Methods section. Please see lines 125-126, 172 and 200.

3) T. cruzi transmission occurs in almost all diverse environments extending from Southern North America to Southern South America. The area focused in this study has many ecological peculiarities, among which, a relatively poor mammal fauna, only three dominant species and one single vector species that does not make large displacements. Two points: How far can authors generalize or what metrics they propose to be used to compare findings across different environments?

A: The reviewer is correct in regard as, in this study, there are a few species (i.e., few mammal species and one vector species) and some of them belong only to this ecosystem. *Mepraia spinolai* is one of the few triatomine species in which adults are not always winged. However, in most triatomine species, population is composed mainly by nymphal stages (Monroy *et al*. 2003, doi: 10.1603/0022-2585- 40.6.800; Sarquis *et al.* 2010, doi: 10.1111/j.1948-7134.2010.00097.x; Grijalva et al. 2012, doi: 10.1186/1756-3305-5-17; but see Noireau *et al*. 2000, doi: 10.1016/S0035-9203(00)90426-7), which, as in the case of *M. spinolai*, are dispersal-restricted, because they can only disperse by walking (Abrahan *et al*. 2011, dx.doi.org/10.1590/S0074-02762011000200019). Therefore, our results are relevant for most life stages of triatomine species, except for winged adults. We believe that triatomine density (total or infected) would be a good metric to compare our findings across different habitats (e.g., rocky outcrops, bromeliads, palm trees), considering the dispersal-restricted feature of most individuals of a colony. This has been previously proposed (Gurgel-Gonçalves *et al.* 2011, doi: 10.1016/j.actatropica.2011.10.010; Ihle-Soto *et al.* 2019, doi: 10.1371/journal.pntd.0007170). In the same line, the density of those mammals coexisting with triatomine bugs would be a good metric to feed the models, since most triatomine bugs would take blood meals from what is offered nearby (Rabinovich *et al.* 2011, doi: 10.1590/S0074-02762011000400016; Gurtler et al. 2014, doi: 10.1371/journal.pntd.0002894; Oda *et al.* 2014, doi: 10.1111/mve.12064). We included part of this information in the Discussion. Please see lines 344-350.

4) What is the evolutionary stage of insects? In other words, what was the population structure of the colonies like? Would this not be a variable to evaluate as well? First stage nymphs for

example move less than more mature nymphs.

A: Unfortunately, there are not many studies regarding triatomines evaluating dispersal by developmental stage (Brémond *et al*. 2014, doi: 10.1186/1756-3305-7-164; Castillo-Neyra *et al*. 2015, doi: 10.1371/journal.pntd.0003433). It is expected, as the Reviewer states, younger nymphs would move less than more mature nymphs. However, non-flying nymphs of wild triatomine species tend to exhibit a sit-and-wait strategy to obtain a blood meal, where prey needs to get close enough to be detected by nymphs before they leave their secure zone (e.g., a place beneath a rock, a rock fissure or inside the roots or leaves of plants), and this stalking behaviour applies to all nymphs as well as adults. In this new version, we are including the population structure of the sampled insects as electronic supplementary material (ESM1 figure S2, and lines 228-229). We agree that this point would be relevant to be examined in future behavioural studies in the field, and in the present manuscript we included this aspect as relevant to be considered and therefore a limitation of our results, probably accounting for part of the unexplained variation. Please see lines 330-333.

5) How to define the variables to carry out comparative studies?

A: We presume the reviewer refers to both predictor and response variables. In both cases, we are convinced that in triatomine-host systems it is better to work with those hosts that would have a high probability to enter in contact with triatomines (higher contact rate), assuming vectors show no preference for specific hosts. This contact rate depends on many factors related to the host life-history traits, foraging behaviour, habitat preferences, territoriality, among many others. If similar biologically relevant factors are included in the models, comparison with other studies would be possible. We also suggest working with different levels of analyses for the response variable whenever possible. Please see additional explanation to this question in points 2 and 3 mentioned above.

6) Preference of triatomines by infected mammals is a trait that should be considered with extreme caution.

A: We agree with Reviewer 1. There are studies showing that there seems to be a preferential feeding on infected mammals (Ramírez-Sierra & Dumonteil 2015) and on immune prey (Hecht *et al.* 2006), all of which would suggest that some animals would be preferred over others, thus increasing the chance of vectorial transmission due to extended contact rate. Such studies, however, were performed in laboratory settings with other triatomine and host species, so we cannot ensure that this would be the case within our wild study system. In any case, the Discussion of the original version of the manuscript already included a sentence about this potential limitation. Please see lines 309-311.

7) As far as I could see, the oral route was not included among the variables. This is a point that deserves at least to be discussed. At least in laboratory conditions, rodents may prey on triatomines or become infected during grooming their fur eventually contaminated by infected triatomine feces.

A: Regarding oral route transmission in mammals, it was not explicitly included since we considered that the eating of infected vectors and the licking of dejections could be indirectly considered as part of the vectorial transmission as well, since it involves the contact between the vector and the host. We had already mentioned briefly this issue in the original version (in the description of the transmission routes in the Introduction, and we mentioned that at least *P. darwini* included insects in their diets). We understand that it might improve the understanding of the article to mention it as a separate transmission path, so we clearly stated this possibility in the Introduction section. Please see lines 72-73. However, since we are modelling infection transmission to vectors, not to mammal hosts, the ingestion of triatomine bugs by insectivorous mammals might have an overall numeric effect on vector colony size. At this point, we cannot assume that this predation would be differential over infected or uninfected vectors, so it was not included as a factor in these models. To be clearer regarding this point, in the Discussion section we stated that in this study *T. cruzi* transmission was directed from mammals to vectors. Please see line 283.

8) Finally, it must be considered that the different T. cruzi DTUs establish different patterns of infection in the different animal species. How could this aspect interfere with authors observations?

A: Certainly, it is possible that different *T. cruzi* DTUs could pose differential transmissibility to vectors. Different host species might be infected with different DTUs (Rozas *et al.* 2007; doi: 10.4269/ajtmh.2007.77.647), and/or may present several DTUs (Botto-Mahan *et al*. 2015, doi: 10.1016/j.actatropica.2015.06.008) with differential circulation in blood, as was reported by Rojo *et al.* 2017 (doi: 10.1186/s13071-017-2314-2). This point was not evaluated in this specific study, but it is an ongoing investigation in our group. Previous studies in the same area have shown that DTUs TCI, TCII, TCV and TCVI are circulating in mammals (Rozas *et al.* 2007, doi: 10.4269/ajtmh.2007.77.647; Botto-Mahan *et al*. 2015, doi: 10.1016/j.actatropica.2015.06.008) as well as in *M. spinolai* (Coronado *et al.* 2009, doi: 10.4269/ajtmh.2009.09-0053). Our study analysed the infection status using primers that are reported to bind to all *T. cruzi* DTUs (Guhl and Ramirez 2013, doi: 10.1016/j.meegid.2013.08.028; Wincker *et al*. 1994, doi: 10.4269/ajtmh.1994.51.771), so we have a broad picture of the transmission of *T. cruzi* from mammals to vectors occurring in that population. This specification regarding the primers was added in the Method section. Please see line 158 and two news references [37,38] in line 159.

Referee 2

1. This manuscript is about the relationship between host diversity (and identity) on the transmission of Trypanosoma cruzi, a protozoan parasite that is the etiological agent of Chagas disease. The effects of hosts were tested against three response variables that came from the only parasite vector in the area, the wingless triatomine bug Mepraia spinolai. The authors wanted to know which of three infection parameters in vectors were more related to host variables. In general, this topic could be of interest of the journal audience. There is a high interest in how is the intensity and direction of the relationship (if any) between biodiversity and disease risk (i.e. vector infection prevalence or density), however, there are very few vector-borne diseases in which this relationship has been studied. As an example, Lyme disease, in temperate ecosystems

is one of the most studied systems, but tropical and subtropical zoonosis remain to be incorporated in a global discussion about how biodiversity can protect people's health. In this sense, I celebrate the attention paid by the authors on Trypanosoma cruzi transmission since Chagas disease is one of the most important neglected diseases in America. However, I have some concerns about the way the authors described their methods and results, mainly regarding the accuracy and precision of their estimations of hosts and vector density. Overall, they calculate density as a simple number of individuals over sampling effort (sampling area for the case of rodents and colony size? in the case of vectors), however, this assumes that for rodent there are no differences in capture probability (for instance, capture-recapture rates) related with behavioral responses toward traps (some rodents are trap lovers while other species are trap haters, etc.), between species or that for bugs there are no biases in collection success. Therefore, I recommend estimating density from models that can incorporate assumptions related to the sampling design. The authors made a very good job collecting a high number of samples in both, vectors and rodents, so I am confident that they can estimate realistic densities.

A: We thank Reviewer 2 for this insightful point. Yes, we calculated density as a simple number of individuals over sampling effort (4-5 days of trapping for small mammals overlapping with the vector colony area; and 1 hour of bug collection per colony in a unit of area used by a vector colony) in a standard sampling area for both small mammals and vector. To clarify this aspect, we added new supplementary material containing a diagram graphically explaining how overlapping small mammals were selected. We also included a new table with rodent densities per year, evaluating differences in capture probability per rodent species. Those estimations were obtained using a robust model for closed captures implemented in the MARK software. Please see ESM1-fig S1 and ESM1-table S4. According to those results, for each year the mean number of each rodent species trapped intersecting the colonies were lower than the total number estimated by MARK for the complete small mammal trapping area, which was expected due to the vector colony-mammal overlapping procedure. It is possible that our density estimations of those mammals associated to vector colonies could be underestimated for some of the rodent species. However, the objective of the methodology was to represent the real host availability for triatomine colonies, considering the dispersal restriction of this vector species. Regarding capture probability, the three most abundant rodent species showed similar capture probabilities (p-hat value ranging between 0.21-0.39), considering the four sampling years, as shown in the ESM1-table S4. In the Results section, we included a sentence indicating part of this information. Please see lines 241-242.

2. Methods: The authors provide very little information about how rodent density was estimated. This is crucial to determine a confident magnitude for predictors. The trapping design is a line-transect or a grid? They set up two lines (transects) in parallel separated by 10 m (100 traps) so this can constitute a grid. What model was used to estimate the density? His was estimated for each species (infected or total) per species/set or over the three sets taken as a whole?

A: We appreciate this comment and we were probably too short with words to keep our manuscript under the maximum number of pages requested by the journal. Yes, we used three grids, each one composed by two 10 m apart-lines of 50 traps each (a total of 300 traps). To improve clarity, we used the word “grid” and we added a supplementary diagram explaining the spatial method to estimate the rodent density per colony. Please see lines 139,141,142,143,178,

179 and ESM1-fig. S1, respectively. Small mammal densities were estimated for each vector colony separately, by the spatial overlapping between the mean area used by a vector colony and the mean area used by each small mammal. This was the inclusion criterion. In addition, in this new version we include density estimations (using the MARK software) for the three most abundant rodent species, per year (the three grids were combined). See ESM1 table S4. Please, see more information in the Answer 1 given to Reviewer 2.

3. Line 198: vector infection probability: the infection status is infected or not infected, therefore, their probability according to the definition of authors will be 1 or 0 per specimen, is that right? How the authors can calculate confidence intervals based on this procedure? The mean of these values over the sampled number is not that the prevalence?

A: The infection status of the insects was in fact binomial (i.e., 0 or 1). The model that predicts "vector infection probability" was a generalized linear model with a binomial error distribution, which uses a logistic regression to obtain the fitted values. To clarify this point, we were more explicit in the parts of the manuscript where the result of this model was depicted. Please see lines 201-202.

4. Results: Line 246-247 These numbers are intervals of confidence at what level? Or is a range considering all the sampling years? (Also this comment applies to Line 226)

A: These numbers are ranges considering all the sampling years. This was clarified in lines 229 and 250.

5. Discussion: Line 268: What authors mean by depauperate? Is this rodent community affected by a disturb (chronic?) that is causing a habitat filtering? Or authors refer to a community with a natural and stable low diversity caused by biogeographic processes?

A: We are grateful to Reviewer 2 for raising this point. We have changed this in lines 75 and 269-271, which we are referring to the second option: a natural and stable low diversity, as result of biogeographic processes that shaped Chilean biota.

6. Line 271-272: What the authors mean for a highly complex dynamics of parasite transmission? It seems that it is dependent on the rodent density which suggests a density-dependent process led by hosts, however, authors did not include vector abundance (or density) which could also have an effect on vector infection if host availability can influence vector competition for blood meals. Additionally, authors did not evaluate which factors could cause the interannual variations on rodents and vectors (temperature, humidity, resources, etc?)

A: We have modified part of this sentence in the text. Please see line 274. Regarding the inclusion of vector abundance in the models, this was evaluated but two of the three tested models presented higher Akaike Information Criterion when vector abundance was included. Please see new information in the ESM1 table S1 and lines 219-220. We included sampling year as a random factor to account for inter-annual variations in temperature and humidity, both factors related to primary productivity and in turn to host abundance. More detailed analyses

with climatic variables are beyond the scope of this study. For vectors, the resources are blood meals obtained from host species, which are included in all the statistical models fitted.

7. Line 277-279: The diversity gradient on this study area is maybe too narrow to observe such a diversity effect if relevant, however, most important in this kind of community, is if the species identity is equally relevant for a vector blood meal and parasite transmission? There were vector colonies associated with different species dominance that also differed in transmission dynamics?

A: The diversity values detected among colonies ranged from 0.360 to 1.171, which were calculated using all the captured small mammal species (not only the three most abundant species included in the detailed analyses). Diversity indices are unitless estimations that account for the number of species and their relative abundances, but have limitations for further inferences upon those values. In our case, species richness is not that high, as the reviewer noticed, but there are changes in relative abundance that are related to changes in H' index, which is accounted for in the statistical models fitted. Regarding the host species identity for blood meals and parasite transmission, it is a topic under investigation and we do not have an answer right now. So far, studies with other triatomine species have noted that there are differences in vector preference (Gürtler *et al*. 2009, doi: 10.1371/journal.pntd.0000447) but to our knowledge there are no studies that evaluate this in endemic triatomine species from Chile. On the other hand, few reports from sigmodontine Argentinean rodents have shown that they have low transmissibility rate of *T. cruzi* to another triatomine species (Orozco *et al*. 2014, doi: 10.1016/j.meegid.2013.12.020). This is the same Subfamily as *Phyllotis darwini*, for which we found a consistent inverse relation between rodent density and vector infection in the three tested models. Other species evaluated in Argentina showed differential transmissibility, with rodents being less infectious to the vector (Orozco *et al*. 2013, doi:10.4269/ajtmh.12-0519). Last, regarding dominance, the SHE analysis showed that there were only two small mammal host species that dominated: *Octodon degus* and *P. darwini*. We did not include a dominance index at the colony level but future studies could test this suggested approach.

8. Line 290-291: This pattern was also reported by Ramsey et al. 2009 Plos One (https://doi.org/10.1371/journal.pone.0046013) in Mexico but the absence of a statistical difference between adult and juvenile T. cruzi prevalence did not support the juvenile dilution hypothesis.

A: We appreciate the Reviewer comments. Unlike Ramsey *et al.* 2009, in our study the juvenile hosts were not tested for *T. cruzi*, as most of them were not accessible by the trapping system due to a weight issue. Therefore, we can only raise this as a possible hypothesis of the mechanism involved.

9. Line 295-296: How the authors statistically compared the three models?

A: We did not statistically compared the three resulting models. As a matter of fact, this is not possible as there are different response variables involved, with different data distributions. What we did was to compare candidate models for each case (i.e., infection density, infection

prevalence, and vector infection probability) and ranked them using the Akaike Information Criterion. Those models are now included in ESM1 table S1. Please see lines 219-220.

Appendix B

RESPONSE TO REFEREE
MS RSPB-2019-3018-R1

Associate Editor
Board Member 1

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

A: All changes highlighted in red in the original version RSPB-2019-3018 were removed. In the new main document uploaded not new changes were included.

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

A: Done as requested.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.
Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

A: Done as requested.

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

A: Uploaded as requested.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

A: Done as requested. Please see lines 361-362.

Reviewer(s)' comments to author

Referee 2

1. I appreciate the attention that the authors paid to my comments/suggestions. I agree with the changes made in this new manuscript and have not further questions. I recommend this paper for publication in this Journal.

A: We appreciate your comment.