Peer Community In Zoology

Welfare threatened species

Peter Galbusera *based on reviews by Maria Luisa Marenzoni and Francis Vercammen*

A recommendation of:

First detection of herpesvirus and mycoplasma in free-ranging Hermann tortoises (Testudo hermanni), and in potential pet vectors

Jean-marie Ballouard, Xavier Bonnet, Julie Jourdan, Albert Martinez-Silvestre, Stephane Gagno, Brieuc Fertard, Sebastien Caron *(2021), bioRxiv, 2021.01.22.427726, ver. 4 peer-reviewed and recommended by Peer Community in Zoology* **10.1101/2021.01.22.427726**

Open Access

Submitted: 25 January 2021, *Recommended: 02 July 2021*

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Peter Galbusera (2021) Welfare threatened species. *Peer Community in Zoology, 100007.* **10.24072/pci.zool.100007**

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Recommendation

Wildlife is increasingly threatened by drops in number of individuals and populations, and eventually by extinction. Besides loss of habitat, persecution, pet trade,… a decrease in individual health status is an important factor to consider. In this article, Ballouard et al (2021) perform a thorough analysis on the prevalence of two pathogens (herpes virus and mycoplasma) in (mainly) Western Hermann's tortoises in south-east France. This endangered species was suspected to suffer from infections obtained through released/escaped pet tortoises. By incorporating samples of captive as well as wild tortoises, they convincingly confirm this and identify some possible 'pet' vectors.

In February this year, a review paper on health assessments in wildlife was published (Kophamel et al 2021). Amongst others, it shows reptilia/chelonia are relatively well-represented among publications. It also contains a useful conceptual framework, in order to improve the quality of the assessments to better facilitate conservation planning. The recommended manuscript (Ballouard et al 2021) adheres to many aspects of this framework (e.g. minimum sample size, risk status, …) while others might need more (future) attention. For example, climate/environmental changes are likely to increase stress levels, which could lead to more disease symptoms. So, follow-up studies should consider conducting endocrinological investigations to estimate/monitor stress levels. Kophamel et al (2021) also stress the importance of strategic international collaboration, which may allow more testing of Eastern Hermann's Tortoise, as these were shown to be infected by mycoplasma.

The genetic health of individuals/populations shouldn't be forgotten in health/stress assessments. As noted by Ballouard et al (2021), threatened species often have low genetic diversity which makes them more vulnerable to

diseases. So, it would be interesting to link the infection data with (individual) genetic characteristics. In future research, the samples collected for this paper could fit that purpose.

Finally, it is expected that this paper will contribute to the conservation management strategy of the Hermann's tortoises. As such, it will be interesting to see how the results of the current paper will be implemented in the 'field'. As the infections are likely caused by releases/escaped pets and as treating the wild animals is difficult, preventing them from getting infected through pets seems a priority. Awareness building among pet holders and monitoring/treating pets should be highly effective.

References

Ballouard J-M, Bonnet X, Jourdan J, Martinez-Silvestre A, Gagno S, Fertard B, Caron S (2021) First detection of herpesvirus and mycoplasma in free-ranging Hermann's tortoises (*Testudo hermanni*), and in potential pet vectors. bioRxiv, 2021.01.22.427726, ver. 4 peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.01.22.427726

Kophamel S, Illing B, Ariel E, Difalco M, Skerratt LF, Hamann M, Ward LC, Méndez D, Munns SL (2021), Importance of health assessments for conservation in noncaptive wildlife. Conservation Biology. https://doi.org/10.1111/cobi.13724

Reviews

Toggle reviews

Revision round #2

2021-05-27

Author's Reply

Download author's reply (PDF file)Download tracked changes file

PCI in Zoology 30 June 2021

Dear Peter Galbusera

This covering letter supports the second re-submission of our manuscript entitled « First detection of herpesvirus and frequency prevalence of mycoplasma infection in free-ranging Hermann's tortoises (Testudo hermanni), and in potential pet vectors » for recommendations in PCI Zoology.

Again, we would like to thank you and both reviewers, for the positive and constructive feedback to improve the manuscript. We agree with all the comments and we modified the manuscript accordingly. As recommended by Mr Vercamenn, we revised the interpretation of the literature cited. As recommended by Maria-Luisa Marenzoni we corrected technical details and revised results, we include additional statistics and rewrote this paragraph.

In the pdf file, we provided a detailed answer to each query

Yours sincerely

Jean-Marie Ballouard Xavier Bonnet.

Decision round #2

Dear Authors,

Both reviewers have commented on the (major) revision text. They still recognize the importance and relevance of the submitted manuscript. They also appreciate the improvements made throughout this revision. However, they also still identify some points of disagreement and aspects that need improvement before this manuscript revision can be recommended.

It is especially important to spend additional attention toward technical details and the correct interpretation of reference literature. Furthermore, the manuscriopt could be improved with some basic statistical analyses (estimating the significance of the frequency differences between groups).

I agree with the reviewers and therefore, I would suggest the authors to revise again their manuscript, taking into account (and individually addressing) all the comments/questions of the reviewers (see two pdfs with their comments).

Download recommender's annotations (PDF)
Preprint DOI: ***10.1101/2021.01.22.427726***

Reviewed by Francis Vercammen, 2021-04-27 19:19

Dear recommender, Peter,

I have made some additional suggestions to improve this manuscript (see PDF file).

Greetings,

Francis

Download the review (PDF file)

Reviewed by Maria Luisa Marenzoni, 2021-05-23 08:05

Download the review (PDF file)

Revision round #1

2021-03-01

Author's Reply

Download tracked changes file
PCI in Zoology 09 April 2021

Dear Peter Galbusera

This covering letter supports the re-submission of our manuscript entitled « First detection of herpesvirus and mycoplasma in free-ranging Hermann's tortoises (Testudo hermanni), and in potential pet vectors » for publication in PCI Biology.

We would like to thank you and both reviewers, Francis Vercamenn and Maria-Luisa Marenzoni, for the abundant, positive and constructive feedback to improve the manuscript. We agree with all comments and modified the manuscript accordingly. We have notably modified the title as recommended by Mr Vercamenn. We revised results (significance level in the text) as well the 3 tables. As suggested by Maria-Luisa Marenzoni, we merged the table with sample with table with the result. We provided more details given diagnostic aspects. The discussion was strongly modified and streamlined. Finally, we revised the references list (corrected and placed in the alphabetical order, news references were also included).

Below we provided a detailed answer to each query

Yours sincerely

Jean-Marie Ballouard Xavier Bonnet.

*** Reply to Reviewer 1 - Francis Vercamenn ***

Query: Line 2 and 231: Mycoplasma has already been detected in free-ranging Hermann's tortoises: 11.8% (11/93) were positive in ELISA [Untersuchungen zum Vorkommen von Mykoplasmen und Herpesviren bei freilebenden und in Gefangenschaft gehaltenen Mediterranen Landschildkröten (Testudo hermanni , Testudo graeca graeca und Testudo graeca ibera) in Frankreich und Marokko – PhD thesis 2003 K. Mathes - Klinik für Vögel, Reptilien, Amphibien und Fische - Justus-Liebig-Universität Giessen].

Answer: We thank reviewer#1 for pointing out this oversight, now corrected throughout the manuscript. Note that we used PCR instead of Elisa.

Query: 1 animal was positive in Mycoplasma PCR (see reference Lecis et al. 2011 on line 417).

Answer: we emphasize that we clearly distinguish native free-ranging individuals from other categories. In Lecis et al. 2011 the tortoise tested positive was kept in enclosure before sampling (Centro Fauna Bonassai, Sassari, Italy), and thus cannot be considered as a native free-ranging tortoise.

Query: Line 35-36: see comment of line 210-221 on the percentages

Answer: checked, modified and corrected as requested.

Query: Line 41: insert "and" between "high" and "should"

Answer: corrected.

Query: Line 56-59: reference DiGeronimo et al. 2019 does not corroborate your statement that Corua bourreti is impacted by URTD, as this references states “The clinical significance of mycoplasmosis in Cuora bourreti is unknown. Although all the animals examined exhibited nonspecific signs of illness, such as anorexia and poor body condition, none exhibited signs usually associated with clinical mycoplasmosis in other chelonian species, such as blepharitis, conjunctivitis, palpebral edema, or mucopurulent oculo- nasal discharge.”

Answer: we fully agree, the text was modified accordingly.

Query: Line 73-75: the reference of Spielman et al. 2004 does not mention anywhere “phenotypic diversity“ and “demographic resistances to diseases”.

Answer: we added a textbook reference (Franckam et al. 2002à). Regarding Spielman et al.’s pioneer study (2004), although performed on different populations of Drosophila melanogaster and despite huge variations, it experimentally demonstrated that loss of genetic diversity significantly reduced resistance to disease. These results support our statement that “*** and genetic depression often hinder physiological and demographic resistances to diseases”. Therefore we believe that this reference is also appropriate.

Query: Line 108-110: the reference of Mathes et al. 2001 does not say anything about mortality.

Answer: corrected.

Query: Line 133: replace “pet” with “captive (pet)”

Answer: corrected.

Query: Line 139: rephrase “and vagrant tortoises (wild exotic pet)” into “and vagrant (wild pet) tortoises”

Answer: corrected.

Query: Line 189-191: please clarify which test you mean with “This test”. Do you mean the combination of PCR and SN? The cited references of Origgi et al. 2001 and Origgi 2012 do not state that PCR has a high sensitivity and specificity.

Answer: Clarified with appropriate reference (Salinas et al. 2011)

Query: Line 210-221: It is not clear how all these percentages were calculated. Please clarify by giving the number of the numerator and the denominator of the fraction.

Answer: this problem was also pointed out by reviewer#2. We merged table 2 and 3 and provided the information requested.

Table 2 is confusing: most individuals were tested for both pathogens TeHV+Myc (421/572), but only 400/572 for TeHV?

Answer: we thank reviewer#1 for pointing out this mistake, now corrected.

Query: Line 215: the number 2.9 % does not appear in table 4.

Answer: corrected.

Query: Line 222: 23/42 = 56.1 % females and 18/41 = 43.9 % males.

Answer: corrected.

Query: Line 224-225: 28 individuals with clinical symptoms of URTD: 4 + 8 + 14?

Answer: corrected. Two individuals with clinical signs were omitted.

Line 234-236: what are the real Mycoplasma infection figures of captives and vagrants and why are these interpreted as high? (see also comment on line 278).

Answer: clarified and the sentence was modified.

Query: Line 239-240: please clarify these percentages.

Answer: corrected.

Query: Line 245-247: the cited reference of Kolesnik et al. 2017 states that herpesvirus was detected in 17% of the animals, but these authors use the reference of Martel et al. 2009 (Reintroduction of clinically healthy tortoises: the herpesvirus Trojan horse. J Wildl Dis 2009; 45:218–220) which revealed 16.3 % detection by PCR. I suggest to use the Martel reference instead of Kolesnik. Ok Also: insert “by PCR” between “detected” and “in”; replace “16 %” with “16.3%”.

Answer: corrected.

Line 248: insert “by PCR” between “tested” and “more”; replace “more than 1,000” with “1,015”.

Answer: corrected.

Query: Line 250: replace “more than 40 %” with “42.1 %” and “8 %” with “8.0 %”

Query: Line 251: replace “viruses (notably herpesvirus)” with “herpesviruses”

Query: Line 253: insert “(SN test)” between “seroprevalences” and “were”

Query: Line 254-255: replace “and ~5% for two other serotypes (X and reovirus)” with “5.2 % for picorna-like ‘X’ virus and 4.9 % for reovirus”

Query: Line 255: replace “Similar results were” with “A similar PCR result of 36.7 % was”

Query: Line 256: I suggest to remove the reference of Soares et al. 2004, as their PCR result was 15.8 % and this is in my opinion not similar to 42.1 % reported by Kolesnik et al 2017.

Answer: corrected.

Query: Line 258: the reference of Marschang et al. 2009 is not in the list of references

Answer: this reference were deleted

Query: Line 258-259: the reference of Hidalgo-Vila et al. 2020 does not say anything about individuals intercepted during illegal trade, as they used established populations in ponds for their study. They indeed found a high prevalence of 35/44 animals with a wide range of pathogens, but only 5/44 animals with Mycoplasma and 5/44 with herpesvirus.

Answer: we preplaced this inappropriate reference with the more adequate Brianti et al. 2010.

Query: Line 261-263: the reference of Mathes et al. 2001 does not say that the free-ranging animals were negative for herpesvirus.

Answer: Mathes et al. 2001 did not found positive test for herpes virus in free-ranging tortoises, but reported a case from captivity.

Query: Line 275: insert “believed to be” between “is” and “horizontal”

Answer: corrected.

Line 278: Kolesnik et al. 2017 state a prevalence of 42.1 % Mycoplasma and 8.0 % herpesviruses: do you refer to this as a very high prevalence of infection for both Mycoplasma and herpesvirus? (see also comment for lines 234-236)

Answer: The discussion was strongly modified, and hopefully ambiguities removed.

Query: Line 317: replace ‘”HeHV” with “TeHV”

Query: Line 318: insert “sperm” between “feces,” and “and”; replace “are” with “can be”

Query: Line 522 and 532: change “Captive” to “Captive (pet)”

Answer: corrected.

*** Reply to Reviewer 2 - Maria-Luisa Marenzoni ***

General comments:

The manuscript entitled “First detection of herpesvirus and mycoplasma in free-ranging Hermann tortoises (Testudo hermanni), and in potential pet vectors” is a very interesting and complex study, that underlines the relevance of sanitary protocols in managing populations of testudos, both free-ranging or captive.

Answer: we thank reviewer#2 for these encouragements.

It is not easy in multidisciplinary studies like this to be understandable because various specialized languages must be used, yet there is a need for the message to reach several scientific fronts. For this reason, I believe that the manuscript can be improved to increase its scientific level and transversality. For example, I am interested in the diagnostic/microbiological aspects and I need more details on this part.

Answer: we modified both the material and method and discussion section, notably to provide further details regarding diagnostic. We provided news reference end discuss. Indeed, reviewing the well documented studies performed in captivity was out of scope of the current study. Yet, if expressly requested by the editor we can include such a review, but this will lengthen the paper.

A general improvement of the English language may be appropriate using a professional service. Another aspect is that, while I share the comments of the authors, many of these are not strictly related to the results, so they should be shortened widely.

Answer: the text was checked and modified. The discussion has been streamlined in order to fit more tightly with the results.

Tables could be improved.

Answer: the tables have been deeply modified as requested.

Specific comments

Queries:

Line 41: "…are high AND should be SCREENED…

Lines 51, 65: sp. is spp. (and not italic)

Line 94: correct in "sporadically"

Line 97: origins instead of provenances

Line 103: approximately instead of the symbol

In general, there is an overuse of words completely capitalized for with no reason (SOPTOM, VIRCON-Virkon, TERUMO NEOLUS, etc.).

Line 149: I suggest to clarify for non-expert "Each tortoise was measured by strait carapace length (SCL),…

Line 150: "nearest gram"

Line 151: "100 mm in SCL"

Line 166: et al. not in italic

Line 172: 1500 rpm FOR 5 min

Line 188: …antibody responses by serum neutralization test (SN)…

Line 191: …DNA of pathogens…

Line 195: the word TeHV already indicate herpesvirus; cut Herpesvirus.

Line 195: Origgi (one r)

Lines 196-197: the acronym PCR has been already introduced at line 188; the single acronym PCR is enough

Line 198:" …detect active infection by Mycoplasma spp. and TeHV"

Answer: corrected.

Line 207: the order of the references is correct? It is not in alphabetical or chronological order.

Answer: we organized the reference in alphabetical order.

For the results, the indication of the period in which the sampling was performed could be useful; some periods, like post-hibernation, is considered a period with immunodepression.

Answer: this information is now provided.

Line 210-212: you can short inserting 7 free-ranging WHT (6 adult females and 1 adult male) were…

Line 213: were positive for mycoplasma DNA

Line 217: nine or 9 in number (based on the guidelines of the journal)?

Line 226-227: tested positive for mycoplasma and negative for TeHV?

Line 227: Tortoise or tortoises?

Lines 233-234: SN and PCR positive could be due to reactivation, not recent infection.

Answer: corrected.

The discussion, in general, are too comments poorly linked to the results.

Answer: as exposed above, the discussion was strongly modified and shortened.

Lines 236-240: on what results do the authors believe that pet animals are a risk for free-ranging? From the tables it appears that free-ranging are more positive than the others.

Answer: this section has been removed from the discussion. Results are now better presented in the discussion, suggesting that pets might represent a risk (although we have no proof, and hence remain prudent). However, based on extensive literature in animals in general and in chelonians specifically, we posit that pet tortoises represent a sanitary risk ("risk" dos not equal "certainty") to free-ranging populations. Many pathogens cross species; contamination levels are particularly elevated in captivity, and cases of transcontinental spreading of extremely dangerous diseases were pet trade was involved are now well documented (beginning of the introduction).

Table 2 and 3 could be merged and the introduction of the percentages for each group could be useful to understand and recognize the group. It is difficult to recognize in the text who are 18% and 40%.

Answer: we agree and modified all tables.

Line 241: TeHV (capital letter for v)

Line 242: I lost the words after “in cages or in….”

Line 244: freeliving by various authors, is it appropriate a citation.

Answer: we better explain our meaning here and modified the text. We highlight that individuals maintained in outdoor enclosures (captive) are not free-living animals experiencing natural conditions (free-ranging). The objective of enclosures is to keep animals captives.

Line 258: cut e.g. in the list of references

Lines 274-286: to surely define the vagrants, genetic profile should be performed. In general, this paragraph is too long and not supported by solid results.

Answer: we agree, this part was strongly modified.

Line 317: TeHV (no HeHV)

Line 321: may promote virus reactivation from latent infection.

Answer: corrected.

Decision round #1

Both reviewers recognize the importance and relevance of the submitted manuscript/research. However, they also pinpoint several issues (in format but also in content/scientific robustness) that need improvement before this preprint can be recommended:

- Results: contains some incorrect or unclear numbers; incomplete at places (e.g. diagnostic/microbial results)

- Discussion: in places too poorly linked to the results (some claims are not supported by results)

- References: incomplete listing, incorrect references (several statements could not be found in the cited papers)

- English language: need special attention by a native speaker

- Minor editorial comments

I agree with the reviewers and therefore, I would suggest the authors to revise their manuscript, taking into account (and individually addressing) all the comments/questions of the reviewers. The reviewers are expecting clear explanations and answers to each issue raised (see two pdfs with their comments).

*Preprint DOI: **10.1101/2021.01.22.427726***

Reviewed by Francis Vercammen, 2021-02-14 18:01

Dear recommender, Peter,

I have made suggestions and asked questions to improve this manuscript (see PDF file).

Greetings,

Francis

Download the review (PDF file)

Reviewed by Maria Luisa Marenzoni, 2021-03-01 16:27

Download the review (PDF file)