

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.

Below we provided a detailed answer to each query

Yours sincerely

Jean-Marie Ballouard Xavier Bonnet.

\*\*\* Reply to Reviewer 1 \*\*\*

First detection of herpesvirus and frequency of mycoplasma infection in free-ranging Hermann's tortoises (*Testudo hermanni*), and in potential pet vectors (Ballouard J-M et al.)  
R1

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.

I disagree with your interpretation:

- Lecis et al. 2011 state: "In this study, all individuals were sampled upon arrival at the center; therefore, local bacterial transmission through direct contact with already housed chelonians is unlikely." The origin of the PCR-positive animal was Arzachena, about 100 km from the centre in Olmedo. In my opinion, this animal was sampled before it was kept in an enclosure in the centre and was not infected by another animal in the centre.

*Answer: in the paper cited details are not available. We have no precise information regarding transportation condition (were boxes and material etc. sterilized?), or regarding how much time elapsed from capture, captivity, and actual sampling. Overall, there is no mean to ensure that contamination did not occur in captivity. More generally, in previous studies the terms free-ranging or wild were used for individuals captured in the field but maintained for various periods in captivity. In the absence of accurate reliable information, we humbly prefer to maintain our prudent interpretation and to clarify the terminology.*

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. Hopefully the revised text clarified this issue.*

I disagree with your interpretation: it is not clear in this reference which percentages belong to the captive or free-ranging group

*Answer: We agree, the reference does not provide the readers any information to calculate percentages of captive vs free-ranging groups. However, the author Karina Mathes performed most of the sampling in our structure (Station d'observation des Tortues et de leur Milieu) and was mainly funded by SOPTOM; thus, we have access to the data base. We checked the data, and we can assert that no tortoise was tested positive to the herpesvirus in the wild. All positive cases were found in exotic tortoises (N=62, Testudo g. greaca=39; T. g. iberica =23) kept during prolonged periods (months, years) in enclosures, or from various origins (e.g., gardens) and recently hosted at the center (N=28, Testudo g. greaca =21; T g. iberica=7). Regarding free-ranging tortoises sampled in the field, 108 native T. h. hermanni and 40 introduced T.G.G. were tested; none was positive to the herpesvirus.*

P 3 line 59: the reference of Jacobson et al. 1991 is not in the list of references

*Answer: we added this reference in the reference list.*

P 4 line 91: Frankham not Franckam

*Answer: Corrected*

P 10 line 287: 8.7.5% should be 8.7%

*Answer: Corrected*

P 10 line 295: 9.6% should be 9.7%

*Answer: Corrected*

P 10 line 306: Mathes et al. 2001 is not corroborating your statement "... only one case known .... natural populations..."

*Answer: Because this statement can be confusing (see answer above), we do not know how to handle it. We can provide details, but this will lengthen the manuscript and cut the flow of ideas.*

P 11 line 376: the number for free-ranging WHT =  $34/391 = 8.7\%$  according to Table 2

*Answer: corrected*

P 12 line 378-380: "... some species to form resistant biofilms in vitro ...": this is only speculative, as the reference of McAuliffe does not include *M. agassizii*

P 12 line 380-381: I suggest rephrasing; e.g. "If *M. agassizii* would be able to form resistant biofilms in the environment, this may contribute to its persistent circulation even under ..."

*Answer: we agree and tuned down this statement as suggested.*

P 12 line 552: delete "more than"

*Answer: corrected*

P 12 line 557: delete one "5.2%" and "(X and reovirus)"

*Answer: corrected*

P 12 line 559-561: the reference of Brianti et al. 2010 mentions that "... infected by ticks, which can be infected by ..." and "... *Hyalomma aegyptium* has been implicated in the transmission of ...", but they did not actually examine their ticks for the pathogens in their study. Thus, this reference is not corroborating your statement "... a wide range of pathogens has been observed in captive chelonians..."

*Answer: We deleted this ambiguous sentence.*

P 13 line 596: the reference of Jacobson 1994 describes herpesvirus in 1 captive desert tortoise and 1200 imported Argentine tortoises. Thus, this reference is not corroborating your statement about free-ranging chelonians in North America

*Answer: we deleted this reference.*

P 13 line 596: delete "Berish et al., 2000"; this reference is not in the reference list either

*Answer: we deleted this reference (which was in the reference list).*

P 20 line 782: this reference of Marschang et al. 2009 is not in the text

*Answer: deleted.*

P 21 line 807: this reference of Origgi et al, 2001 is not in the text

*Answer: corrected.*

P 26 line 878 Table 2:

- $7/400 = 1.8$  not 1.7
- $4/11 = 36.4$  not 36.3
- $52/560 = 9.3$  not 9.2
- twice: 1(100) not (100)

*Answer: corrected.*

P 27 line 881: delete  $N=457$  so that it reads "... infections of free-ranging tortoises in ..."; the total number of 457 is confusing in this table, as the total number of tests is 300 and 448 for TeHV and *Mycoplasma*, respectively

*Answer: We rewrote the sentence as suggested.*

\*\*\* Reply to Reviewer 2 \*\*\*

The manuscript entitled “First detection of herpesvirus and frequency of mycoplasma infection in free-ranging Hermann tortoises (*Testudo hermanni*), and in potential pet vectors” has been improved.

It is very informative; however, some limitations are still present, especially reading from a specialist point of view, in which technical details are required. Relevant data is there but the technical language is not always appropriate. It is difficult to write a multidisciplinary manuscript because each language generally has very technical aspects.

*Answer: We thanks the reviewer for this encouragement, modified the text, and hopefully improved it. One of the authors is a veterinarian (MSA), and its contribution was crucial to use appropriate technical terms.*

There is no statistical analysis that compares the frequencies of the groups. However, the data allows any readers to calculate these differences.

*Answer: We added statistical analyses as requested; hopefully, this section has been significantly improved and is more clear.*

The authors should uniform the capital letters or not when writing the name of the etiological agents.

*Answer: corrected*

Perhaps it should be clarified in the introduction that infection is not the same as disease and that respiratory forms can also be triggered by other etiological agents while here only two of these, probably the main ones, are evaluated.

*Answer: we modified the introduction as requested. We note however that many causes can provoke respiratory diseases, including noninfectious ones (e.g., heavy smoke during fires), but this issue is out of scope of this study.*

### **Specific comments**

Line 2: “frequency of mycoplasma infection...” generally is prevalence of infection.

Corrected

Line 34-35: it is important to identify the test to diagnose an infectious disease: “by PCR” in these sentences. On the contrary the samples were negative for TeHV by SN.

*Answer: we included this information*

Line 42: “screened” instead of “scrutinized”.

*Answer: corrected*

Line 62: “on agents of URTD” is better than “on URTD” because the study investigated some etiological agents of URTD, not only the disease. In this perspective, statistical analysis would be useful.

*Answer: modified as requested, novel statistical test included.*

Line 71: here Herpesvirus could be written without capital letter because it is written as normal word and not in Latin for taxonomy.

*Answer: corrected*

Line 182: "et al." not in italics.

*Answer: corrected*

Line 203: TeHV type 1 and 3, not serotypes.

*Answer: corrected*

Lines 207-219: the reference of Soares et al., 2004 for the protocols of PCR is correct, but he used protocols previous published in other studies; please, also indicate these protocols to facilitate the rapid identification of the protocol to the reader. This is relevant to compare sensitivity and specificity of different tests.

*Answer: We fully agree. We added these two important references.*

Lines 220-221: data of the Table 2 is pertinent of results. It should be moved at the beginning of the results.

*Answer: done.*

Lines 235-244: in my opinion this part is more redeable by the table 2 and the text could be cut.

At the end of the results a statistical comparison among groups would have been useful to identify risk or protective, or not relevant factors.

*Answer: modified as requested.*

Line 257: why contamination and not infection?

*Answer: corrected*

Line 272: contamination or transmission?

*Answer: corrected*

Line 333: I suggest full stop and newline with the word "Further...".

*Answer: corrected*

Line 365: spectrum, range, instead of spectre.

*Answer: corrected*

Line 385: URTD, insert the T.

*Answer: corrected*

Line 606 and 614: Results in capital letters at the beginning of the caption of the tables 2 and 3.

*Answer: corrected*

Table 2 and 3: the symbol “+” could be misinterpreted. I suggest to write “positive results” “positive test” or similar.

*Answer: corrected.*