Dear recommender,

According to the recommenders' and the reviewers' comments, we are submitting a new version of our manuscript entitled « **Experimental design impacts the vector competence of Ornithodoros ticks for African swine fever virus: a meta-analysis of published evaluations** ».

Attached is the revised version with the track change mode (we refer to this version when citing lines in our replies). You will also find a clean new version on BioRxiv (LINK).

We would like to thank you and the reviewers for their constructive comments on our preprint. Detailed responses can be found below. We hope that the changes we have made following reviewer's queries and suggestions have improved the quality of the manuscript and now ensured its appropriateness for recommendation in PCI Infections.

Recommender: Jean-Mathieu Bart

After the review process, the manuscript was positively evaluated by the two reviewers, both experts in the field of virology.

The effort made by the authors to carry out an exhaustive analysis of the literature was appreciated, with a well-written MS, a relevance to the topic due to the need to standardize experimental protocols in order to better compare the results obtained in different laboratories.

Some minor points that could easily be corrected were pointed out (such as the absence of italics, the presence of 2 in Figure 2, the absence of some references, etc.). In particular, one of the reviewers suggests shortening the MS by eliminating repetitions that may occur throughout the text. A new shorter version of the MS will give more impact to this work.

We tried to shorten a bit the manuscript and we reorganized it so that it is easier to read.

Even though the MS "provides recommendations on how to refine experimental studies to minimize bias and obtain more comparable and reliable results on the vector competence of Ornithodoros for ASFV", the authors are asked to propose "a clearer proposal of a well-standardized procedure for future vector competence studies".

We now provide clear recommendations for standardized protocols in the conclusion section (L 467-480)

In conclusion, this work is highly relevant and deserves publication, but would be of greater interest if it could provide more practical procedures to help researchers in this field to standardize experiments across laboratories.

by Jean-Mathieu Bart, 27 Oct 2024 19:34
Manuscript: https://doi.org/10.1101/2023.11.10.566648
version: 3

Review by anonymous reviewer 1, 22 Oct 2024 11:46

The manuscript PCI Infections #235 by Jennifer Bernard et al is a systematic review of the literature available from 1960 up to now about the vector competence of Ornithodoros ticks for ASFV strains. Three consecutive steps of the tick competence were globally considered: i) tick infection behind the midgut measured at least 61 days post exposure to distinguish ASFV replication from residual

virus from blood meal or inoculation ; ii) horizontal transmission of ASFV to pigs ; iii) tick-to-tick transmission of ASFV through sexual or transovarial route. From 246 references selected (reviews, original manuscripts, thesis) 39 references presenting original results for vector competence were selected including 5 natural tick infections, 34 laboratory experiments on ticks and 2 mixed papers. This results in 51 tick-virus associations between 10 tick species and 38 ASFV strains. The semi-manual, semi-statistical analysis of the data shows that the influencing parameters which mostly impact the evaluation of the vector competence in ticks/ASFV are : i) the titre of tick inoculum, ii) the tick stage, the late stages (nymph 4 to adults) transmitting a higher blood volume with higher ASFV titre ; (iii) the inoculation method of the tick (natural or artificial). In conclusion authors advise on establishing and respecting standards to reduce experimental biases for future investigation of vector competence.

This exhaustive review obviously consists of a significant effort for collating the very diverse literature on vector competence in ticks/ASFV. It plaids for experimental standardisation in order to extract more significance by comparing « comparable » results. Even if interesting bias are clearly outlined and some interesting data are emerging in term of several vector/virus couples, one could have expected a clearer proposal of a well standardized procedure for future studies on vector competence.

As stated above, we now provide clear recommendations for standardized protocols in the conclusion section (L 467-480)

The manuscript itself looks correctly written:

- **It could have been eventually shortened (simplified) from place to place to eliminate repetitions while keeping impact.**

We tried to eliminate repetitions wherever it was possible.

I am not a specialist of all the "material and methods" sections and was a bit not competent across them.

- **the first part in the discussion is interesting in trying to support the differences observed through the studies by differences in biological/pathological/immunological features already observed in the listed references.**
- **Some of the references seems rather old but this is the task of the review to re-visit > 50 years of literature and fig 2 shows that the publication stream was rather fluid except some "holes" possibly depending on technical bottlenecks.**

Let me outline punctual remarks/questions which may clarify improve the work:

- **Lines 36 : Asfaviridae in italics.**

We changed it.

- **Line 38 : natural vertebrate (or mammals ?) reservoirs. By the way yes, ASFV is a porcine pathogen but would it be significant to mention somewhere that ticks can feed on other mammals and eventually transmit (I am not a specialist and I don't know if it is true)**

Actually, only suids are described as vertebrate reservoirs of ASFV. Following your comment, we decided to add 2 sentences about alternative tick hosts at the end of this paragraph

L51: "Ornithodoros soft ticks feed not only on wild suids but on a large variety of vertebrate hosts (aardvark, porcupine, chicken tortoise…). However, the presence of ASFV in these non-suid hosts has never been detected (Thomson, 1985) ."

We also modified another sentence where "vertebrate animals" where mentioned but only suids were concerned. Therefore, we replaced "vertebrate animals" by "suids".

L109 "Regarding virus, we recorded the strain, the genotype (on the basis of p72 gene sequences, Qu et al. 2022), the geographical origin, and the host (suids or ticks) from which the ASF virus was isolated"

- **Line 44 : "more recently". When?**

We added this information

L49: "as it occurred in the Caucasus in 2007 or in the Caribbean in 2021"

- **Line 81 : Why only in English ? Is it not interesting PhD / reports in other languages? What is a "high publication standard" ?**

We changed this sentence to give explanation for this choice:

L88. "For practical issues, we decided to consider only resources published in the English language."

We deleted the expression "high publication standard" which was not straightforward and explained later in this paragraph that:

L95: "Additionally, reviews lacking original vector competence results and offering merely descriptive information were excluded. In addition to scientific articles published in peer-reviewed journals, PhD manuscripts were also selected."

- **Line 90 : "at the end" or "in" ?**

We decided to change the sentence since it sounded a bit strange. We now state:

L 100: "Finally, a review of the citations in the included references was carried out in order to identify further relevant studies. "

- **Lines 99-100 : I am a bit concerned by the necessity and the condition of designing "experts". One unique work may be realized once in life by an illuminated person… Yes, longevity in a topic is usually a proof of competence but this review is not for attributing medals…**

We totally agree with that. The objective was to highlight the fact only few people really work on this topic.

We change the sentence in this paragraph and another on in the results section to show that we're not interested in the experts themselves but rather on the expert community and its limited size.

L109: "General bibliometric information on authors and years of publication was collected to describe the general interest in tick vector competence for ASFV and to identify the community of scientific experts"

L257: "The community of scientific experts identified is rather limited with only thirty-nine authors listed as first and last authors of the selected publications"

- **Lines 101-102 : how authors solved this challenge ?**

Actually, this sentence was a bit misleading. We explained in deep, in the next two paragraphs how we dealt with incomplete and missing data for virus strain (L 114-123) and tick species (L124-133). Therefore, we decided to delete this sentence.

- **Line 103 : Qu et al. 2022 is absent from the reference list**

This has been corrected.

- **Line 130 "associated" or "used" ?**

As suggested, we modified it with "used".

- **Lines 130-133 : the definition of "inter-tick transmission" (direct-indirect) looks to me a bit large and could merit two words for explanation.**

Thanks for the advice. We developed a bit the notion of inter-tick transmission.

L 145: "Similarly, detection of ASFV in tick sexual mates, offspring confirms sexual and vertical transmission. The transmission of virus between ticks via co-feeding has been described in hard ticks, but this phenomenon has not been demonstrated for ASFV in soft ticks (Pereira de Oliveira et al., 2020)"

- **Line 206 : the shown fig 2 is not a "flow chart"**

We changed this sentence and now mention figure 2 and infection status to help the reader understand this ranking.

L 233: « "score 0" for ticks that were unable to become infected or for which it was impossible to conclude (IS = 0 or IS = NA, figure 1) »

- **Lines 208-09 + 215-16 : ASFV transmission from ticks to other ticks. Would it be necessary to mention « co-feeding » that I did not see across the manuscript ?**

Following your advice, we added a sentence about co-feeding:

L 148: "The transmission of virus between ticks via co-feeding has been described in hard ticks, but this phenomenon has not been demonstrated for ASFV in soft ticks (Pereira de Oliveira et al., 2020)"

- **Lines 235-36 : « The names of first and (or ?) last authors"**

We used both first and last authors names considering that they were both expert in soft tick competence. This is stated in Materials and Methods L 99 and in the legend of Figure 2 (L 239). We modify this sentence to avoid any misunderstanding:

L 257: "thirty-nine authors listed as first and last authors of the selected publications"

· **Line 234 & 266 : there are 2 fig 2 !!!**

Yes, this was a mistake. We corrected that and we now have Figure 2 and Figure 3.

· **Fig 4 : not clear to me what means « virus host » and « tick expo. » ?**

Virus host is the "*the host from which the ASFV strain was sampled*", as stated L 220.

It was not very clear throughout the text so we modified it in the results section:

L340: "The potential geographical co-occurrence of tick and virus, the exposure route for tick infection and the host from which ASFV was isolated were not significantly linked with infection status even if they were present in some of the best models"

In figure 4, we changed "tick expo" into "expos. route" and we modified the legend to help the readers.

L334: « "Virus host" is the host from which ASFV was primarily isolated. "Expos. route" is the exposure route for tick infection (natural, artificial, capillary, injection). »

· **Line 362 : « is in agreement with our results » . Which results ? Are there shown ? Referenced ?**

This was stated in the result section (L309). We decided to add a figure with all infection status for virus strains in Annex 6.

L326: "Among the random effects tested, the tick species and virus genotype had no effect on the infection status but the viral strain had (χ_1^2=4.879 p-value= 0.027, see also Annex 6)."

We also expand a bit on this result in the discussion:

L 412:" This is in agreement with our results which showed significant variation in tick infection status depending on viral strain. However, no effect of virus genotype on tick infection status was found, which may be due to the very imbalanced dataset regarding virus genotype with genotype I being overrepresented compared to other genotypes."

· **Line 396 ; incomplete reference « p. ???".**

We did not find incomplete reference here in the text: Burrage (2013) and Forth et al (2020) seem to be complete.

· **Annex 1 : What is in « scopus » which is not in « pubmed » and vice-versa. Could you better discuss duplicates ?**

Actually, Scopus has a larger coverage and scope than PubMed which means that it includes more journals but also books and books chapters. In the final list of included references, only 6 were not found on PubMed. Duplicates are only articles that were found in more than one bibliographic database.

We give more details in the legend of Annex 1.

L644: "Except for 2 articles, all the articles found in PubMed were also found in Scopus, creating 136 duplicates that were removed. The larger number of references obtained in Scopus was expected since Scopus has larger scope and coverage than PubMed (AlRyalat et al., 2019)".

Review by Pierre ROQUES, 20 Aug 2024 16:39

In this article, J Bernard and co-authors did a very pertinent analysis of the animal-models of the African Swine Fever Virus (ASFV) infection. They did a large litterature analysis on articles that deal with the vector competence of various species of Ornithodoros ticks an did a very carefull examination of the result they re-analyse in comparieson taking in account the protocol designs and thus the viral strains the infectious dose and the infection protocol as well as in rare case the passage through the target warm-blood animal (ie the pig). The bibliography-analysis follows the more accepted common rules in addition to a carefull extraction of all the available data and their validation. It is interesting to note that in few of this published article the viral titration is not clearly assessed that question the reviewers for this specific article.

This article is a "cas d'école" in term of analysis and deserved a large publicity, even if some points remained to be extended and specifically the "valuable insight how the future trial can be refined" as defined in the abstract. Thus, the main suggestions should be included in the abstract like 1) line 424 about the detection of viral DNA, or 2) to not use direct injection of viruses within haemocoel.

We added suggestions in the abstract:

L 29: "Our results call for standardised procedures in vector competence experiments to facilitate further investigation and reduce potential experimental bias. In particular, we recommend the use of late nymphs or adult ticks from a laboratory colony to achieve efficient infection rates. In addition, viral inoculation should be carried out by blood meal rather than by injection, and preference should be given to high titre blood. Finally, detection of viral DNA should be performed 2 months after inoculation to distinguish between successful replication and residual virus in the tick."

About this later, It is important to note that the direct virus injection, if it impairs the vector competence analysis, may provide valuable information about the viral cycle within the insect salivary gland as an example.

This is true so we added this idea in the Mat & Meth section.

L 200: "Inoculation by injection allows accurate assessment of the virus dose received by the tick. As it delivers the virus directly into the haemocoel, circumventing the midgut barrier, it offers valuable insights into the molecular compatibility between the virus and the tick. However, it is acknowledged that the injection can be traumatic for the tick, resulting high mortality and leads to distribution of the virus throughout the tick (Bonnet & Liu, 2012)."

<u>Minor comments:</u>

Line 261: the figure 3 is lacking.

Yes, this was a mistake. We had two figures 2. We corrected that and we now have Figure 2 and Figure 3.

Line 286-287 : is there really 8 years ?? not 8 months

Yes, this very long duration comes from Boinas et al 2011. Ticks were collected Portuguese farms where ASF outbreak occurred. Tick collection was performed from 1 month to up to 6.5 years after

outbreak. Ticks were then kept alive in the lab before ASF viral isolation from tick homogenates. In the case mentioned here, ticks were tested 8 years after outbreak. ASFV was not detected in this case. But the authors were able to detected ASFV in ticks in the same farm, up to 5 years after outbreak. We added some details in the text.

L316: "The longest reported DPE are from Boinas et al. (2011) and represent the duration between an ASFV outbreak and the date of virus detection in ticks in Portuguese farms, with virus detected in ticks up to 5 years after the outbreak."

We also realize that the following statement

L 286 (now 319) "half experiments reported a DPE lower than the duration required to differentiate a successful viral replication inside the tick from a detection of residual virus after the infection"

was somewhat misleading since some of those experiments performed before 61 days post exposure analyzed tick organs or did transmission experiment, allowing conclusion on tick infection status.

Therefore, we added:

L321: "Among them, one third performed virus detection in organs or transmission experiments, allowing conclusion on tick infection status."

Line 296: I suggest to use tick colony type and not "tick colony status" that is a confounding term. If before this sentence in the text it is clear (from the field or the lab), here the reader may think that this is the size of the colony or if it is a long-term moribund stock or a young one with high reproduction efficacy, or as discussed later colony with individuals from different development stage.

Ok, we modified the text and used "tick colony type" throughout the manuscript (L 124, L 220, L 328)

<u>Specific editor questions:</u>

Title and abstract
Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know
Does the abstract present the main findings of the study? [ ] Yes, [X] No (please explain): main suggestion deserved to be provided here even if quite trivial, [ ] I don't know
Introduction
Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know
Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know
Materials and methods
Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know
Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know
Results
In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [X] I don't know : I'm not sufficiently expert in statitics to fully validate the analysis but that is described using the Akaike Information Criteria and weight seems relevant.

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know
Discussion
Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know
Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know