An helpfull "to-do-list" to experimentally study Ornithodoros ticks vector competence for African swine fever virus.

Jean-Mathieu Bart based on peer reviews by Pierre ROQUES ⓘ *and 1 anonymous reviewer*

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Bart, J.-M. (2025) An helpfull "to-do-list" to experimentally study Ornithodoros ticks vector competence for African swine fever virus.. *Peer Community in Infections*, 100235. 10.24072/pci.infections.100235

Bernard and colleagues (2025) propose a deep analyze of the literature from the 1960s dealing with experimental studies on the vector competence of *Ornithodoros* ticks, which are involved in the transmission of African swine fever virus (ASFV).

They focused their analyses on 39 experimental designs and protocols that could influence the data obtained, interpretation and therefore the conclusions drawn, with major implications for knowledge of the epidemiology of ASFV. This meta-analysis is based on a total of 10 tick species associated with 38 virus strains, resulting in 51 tick-virus associations.

From their work, the authors formulate several recommendations, such as (i) using late nymphs or adult ticks from a laboratory tick colony to increase the volume of blood uptake, (ii) using blood meal instead of hemocoel infection to mimic exposure, (iii) preferring blood from viremic pigs or blood with a high viral load to ensure sufficient viral load, (iv) wait 2 months after challenge to assess viral DNA and/or use RT q-PCR to detect active infection by assessing ASFV gene expression.

Overall, the authors advocate a standardization of protocol designs by laboratories to to reduce the potential risk of bias.

This article represents an important progress in the field, and will certainly prove useful to the academic community working on this highly relevant pest. This is why I was glad to recommend this preprint to PCI Infections.

References:

Jennifer Bernard, Sara Madeira, Joachim Otte, Fernando Boinas, Marie-Frédérique Le Potier, Laurence Vial, Hélène Jourdan-Pineau (2025) Experimental design impacts the vector competence of *Ornithodoros* ticks for African swine fever virus: a meta-analysis of published evaluations. bioRxiv, ver.5 peer-reviewed and recommended by PCI Infections https://doi.org/10.1101/2023.11.10.566648

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.11.10.566648
Version of the preprint: 3

Authors' reply, 10 January 2025

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Decision by Jean-Mathieu Bart, posted 27 October 2024, validated 28 October 2024

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.

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".

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.

Reviewed by anonymous reviewer 1, 22 October 2024

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.

The manuscript itself looks correctly written:
·   It could have been eventually shortened (simplified) from place to place to eliminate repetitions while keeping impact. 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.
·   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)
·   Line 44 : "more recently". When?
·   Line 81 : Why only in English ? Is it not interesting PhD / reports in other languages? What is a "high publication standard" ?
·   Line 90 : "at the end" or "in" ?
·   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…
·   Lines 101-102 : how authors solved this challenge ?
·   Line 103 : Qu et al. 2022 is absent from the reference list
·   Line 130 "associated" or "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.
·   Line 206 : the shown fig 2 is not a "flow chart"
·   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 ?
·   Lines 235-36 : « The names of first and (or ?) last authors"
·   Line 234 & 266 : there are 2 fig 2 !!!
·   Fig 4 : not clear to me what means « virus host » and « tick expo. » ?

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

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

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

Reviewed by **Pierre ROQUES** ⓘ, 20 August 2024

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.

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.

Minor comments:

Line 261: the figure 3 is lacking.

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

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.

Specific editor questions:

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