Reviewer's report

Title: Spatial distribution and risk factors of Brucellosis in Iberian wild ungulates

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Reviewer: Jacques Godfroid

Reviewer's report:

The paper summarizes findings related to brucellosis infection and risk factors in some of the most important Spanish wildlife species.

Although being of interest in the context of wildlife diseases, the manuscript needs some major revision.

Wild ruminants

It is important to document that wild ruminants are only spill over and not maintenance host for Brucella abortus and Brucella melitensis. However, there is no discussion about the source of infection of the few culture positive animals in this manuscript. There is no mention on the molecular fingerprints of those strains as to compare to strains isolated from livestock. This information should be provided in order to understand which are the risk factors for wild ruminants to be infected with Brucella sp. More, the authors should explain why only 1 out of the 31 iELISA positive red deer samples was culture positive: what is the positive predictive value of an positive iELISA in these species?

Wild boars

It seems out of (reasonable) reach to list 58 additional variables which eventually were not considered to be risk factors. Some of the variable listed are really far fetched and should not have been considered for inclusion in a sound explanatory model. On the other hand, I am questioning the validity of the findings presented in table 3. Indeed, I have difficulties to understand that brucellosis in wild boar is significantly associated with “road” (for what is means) and with “urban” (for what is means) at the peninsular level. This definitely needs clarification.

I also have difficulties to understand the association between brucellosis and the abundance of Iberian hares: what does this actually mean? I also have difficulties to understand that the presence and numbers of open-air raised pigs and pig farms are risk factors. There are 2 main reasons for this:

1. Indeed it is difficult to accept as suggested by the authors that density is not a risk factor within the wild boar population (which we know are infected), and on the other hand to accept that density of pigs in open air pig farms is a risk factor (no information on the brucellosis status of these farms is provided; reference is made to published study although it would have been of the utmost importance to link temporally positivity in wild boar with positivity in pig farms in this study if one want to address risk factors proficiently). This seems inconsistent and should be
clarified.

2. I do agree that association does not mean causation so the question is: who does infect who? In order to clarify, can the author document any case where wild boars were contaminated by open-air pigs? Wild boars are known to sustain B. suis 2 infection. Actually, this is the rule, so this does not need to be discussed as if this is a new finding.

3. Can you please clarify why some variables associated to open-air pig farms are associated with brucellosis in wild boar and other variables associated to open-air pig farms are not (like open-air pig farms in municipality or open-air pig farm per km2)?

Lastly, it is also very difficult to understand that variables like wild boar management, fencing (which fencing is this? Wild boar or open-air pig farm) and wild boar supplemental feeding were not selected. Indeed, these are variables documented to be associated with brucellosis; more the authors refer to this practice to be significantly associated to (bovine) brucellosis in Yellowstone in the US.

To summarize, it seems that the statistics may be correct but the epidemiology and the biology of the infection are not! I genuinely do think that confounding risk factors are listed while actual ones were overlooked. It would also be very interesting to analyse the trend of the evolution of seroprevalence in wild boars during this long period of time (special and temporal analysis): Rise? Plateau? Cyclic? Indeed this would help in understanding if the management of wild boar is important with regards to brucellosis at the peninsular level. This has indeed a lot of consequences for the competent veterinary authorities in the context of the brucellosis eradication program.

ELISA

The Protein G ELISA has been established since 15 years in wild boars. Surprisingly, the authors refer to publication 56, in which the test is described but unfortunately without referring to it for the set up of the iELISA.

Can you please explain the rationale to use the rationale to use B. melitensis 16 M LPS (M dominant) to test for antibodies raised against B. suis biovar 2 (A dominant).

No data on the optimal dilution of sera, according to the species is presented. More, it seems difficult to accept from a practical point of view to have different working dilutions according to the species to be tested.

Why do the authors use ABTS, which is known not to be the best and most user-friendly chromogen?

There is no information provided for positive controls (degree of positivity in quantitative tests). So, the interpretation grid doe not mean a lot to the reader.

By defining different cut-offs, it is expected that a lot of confusion may be generated. Indeed, if one will test at the same time some wild goats, deer and wild boars (for example), the serum dilution will be 1/100 for the first species and
1/50 for the other species while the cut-off will be 50% of the positive control for the first 2 species and 50% of the positive control for the last one, not mentioning that one has to make sure that the correspondent positive control is correctly chosen.

No information is provided regarding results in other serological tests. This would help to compare and evaluate the iELISA. Indeed, if results are redundant with other tests then one is allowed to question the added value of this iELISA.

To summarize, this system is not practical, may easily lead to errors in dilutions, misinterpretation and last but not least is not validated yet. It is virtually impossible to validate serological tests according to the gold standard in wildlife species. Therefore comparison with established tests or other techniques like latent class analysis are recommended.

Yersinia enterocolitica O:9 (YO9)

Yo9 is known to be a major problem in domestic pigs (cross reactivity in serological tests). To which extend is the problem prevalent in wild boar? Is an infection with YO9 not a likely explanation for the high number of iELISA+/culture–animals? Information related to YO9 is needed in order to hypothesise as the authors do (line 365-375).

General comment

This manuscript is too long but also imprecise and incomplete. Some important issues have to be addressed whereas some redundant and known information do not deserve to be published again in order to recommend this manuscript for publication.

I would recommend to re-writing the manuscript as a short communication highlighting the following:

1. Different wildlife species may be infected with Brucella sp in Spain.
2. Wild ruminants are not sustaining Brucella abortus or B. melitensis infection, while wild boars do sustain B. suis biovar 2 infection and may be a reservoir for open-air pigs.
3. B. suis biovar 2 type A, B and C are found in Spain.
4. Can any recommendation be made to the competent veterinary authorities to manage brucellosis in wild boars according to the identified risk factors?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.