Reviewer's report

Title: Emergence of New Biovars and Sequence Types of Brucella isolates, Inner Mongolia, China

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Reviewer: Moon Her

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'Emergence of New Biovars and Sequence Types of Brucella isolates, Inner Mongolia, China'

Yanfen Chen, Yuehua Ke, Yufei Wang, Xitong Yuan, Xiaoyan Zhou, Hai Jiang, Zhoujia Wang, Qing Zhen, Yaqin Yu, Liuyu Huang, Buyun Cui and Zeliang Chen.

This manuscript describes changes of the etiological agents of human brucellosis in 3 stages, high incidence (B. melitensis biovar 2 and 3 in 1950-1960s), decline (B. abortus biovar 3 in 1970-1980s) and re-emergence (B. melitensis biovar 1 in 1990-2000s). In conclusion, authors describe that “this change is consistent with the increase and decrease of brucellosis incidence”. In another part (page 12, line 22), “Biovar BM1 in 1990-2000s replaced BM2 and BM3 in 1950-1960s. Therefore, this implied a speciation evolution among the Brucella isolates during the three incidence stages.”

But I can’t agree with these insistences of authors. They seem to be confused to differentiate between Brucella species/biovars and sequence typing. The biological and biochemical properties of Brucella species/biovars are not changed easily. Those (classical biotyping assay) are golden standard to classify Brucella species and biovars. However, point mutation of nucleotide is very easier than them, and the change of between biological properties and sequence typing is not connected. Therefore, authors do not make inferences from the change of Brucella species/biovars. There is more connected with input of new strains, outbreaks of animals (include change of population, management e.t.c), improvement of health hygiene and so on.

- Major Compulsory Revisions

1. Page 2, line 4 and 14 : dominant (1970-1980s), is it correct ? Please check again. It change to “decline”

2. page 2, line 19-22 and page 10, line 17 - page11, line 6. These results are natural as according to reference 8 (Whatmore et al, 2007) and is not any new one. B. abortus and B. melitensis is divided into two clusters by MLST. I think that authors have to use data of MLST based on/within Brucella species level.
3. page 3, line 3 : authors insist “Both biovars and sequence type of Brucella strains from Inner Mongolia has been changed in the three stages. This change is consistent with the increase and decrease of brucellosis incidence”. I don’t agree with them. Please change to the reasonable conclusion.

4. page 11, line 16 – 17 and Table 5 : please change table based on Brucella species. It is easily to confirm sequence type (ST) belonged to B. abortus, B. melitensis and B. suis.

5. page 12, line 22 - page 13, line 2 : please delete this sentence. This is not acceptable.

6. page 16, line 14 – 15 ; “This implied that strains from the low incidence stage exhibited a higher diversity than those from high incidence stages.” This results cannot be generalized. Please delete.

7. page 17, line 5 – line 20 ; The results of sequence typing and their relations can be only deduced but do not conclude them. Please re-write the whole paragraph. Figure 2 is also not proving their relation. And please remind that sequence typing have to make an interpretation within Brucella species(abortus or melitensis).

8. page 18, line 13 – 14 : “A singleton strain Bru020 was in close relationship with B. ovis, an ancestor species in Brucella.” I don’t understand. How to give a score or number of aroA, cobQ, glk and gyrB gene. Please explain it.

- Minor Essential Revisions

9. page 11, line 15 : please correct table 4 to table 3.

10. page 15, line 15 -16 ; this sentence, “In 1990-2000s, more B. melitensis were isolated” is duplicated before one. Please delete.

- Discretionary Revisions

11. page 16, line 17 : “ ---- might be the differences in species distribution.” # will be the differences in distribution of species and biovars.

Moon Her, DVM, MS, Ph.D. Senior Researcher
OIE Reference Laboratory for Brucellosis, Bacterial Disease Division,
Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA)

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests