Reviewer's report

Title: Longitudinal monitoring of Ehrlichia ruminantium infection in lambs and kids by pCS20 PCR and MAP1-B ELISA in The Gambia

Version: Date: 22 March 2007

Reviewer: Suman M Mahan

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General
The data does NOT define the specific role of tick transmission in initiating an endemically stable state in indigenous small ruminants exposed to field challenge in The Gambia. The supporting data for this statement is that the earliest tick infestation detected was at Keneba during week 16 of observation. However the first mortality (not clear at which site these were recorded), was detected due to heartwater on days 28, 42, 56, 63, 84, 112, (lambs), day 63, 91 (kids) before any ticks were found on these animals. The first tick infestation was during week 16 of life and by inference heartwater mortalities should have only been observed 2-4 weeks after the first record of infestation. Since all the mortalities were before the ticks were noticed on these animals it puts the issue of tick transmission and endemic stability into a cloud of uncertainty. Secondly, and most important parameter in defining endemic stability, should have been to show that the surviving animals were resistant to live virulent challenge. The data presented does not indicate any such challenge with live E. ruminantium, to demonstrate that they had been previously exposed and were resistant to re-infection. Hence, the objective was not achieved. However, the authors did observe some interesting findings which they described by longitudinally monitoring the infection status of the subject animals.

I suggest that this become the objective of the study and the paper!!

PERTINENT COMMENTS
This paper presents data regarding E. ruminantium infection dynamics of neonates-lambs and kids in an endemic heartwater region of The Gambia. The data addresses some key areas that are undefined in the epidemiology of heartwater in these animal species and for this region. Understanding the epidemiology of infection in neonates would facilitate better control strategies for heartwater. The data presented suggests that lambs and kids are probably exposed to E. ruminantium infection in early life either through in-utero transmission (vertical) or via colostrum or ticks and also suggests an inverse relationship of age with susceptibility to heartwater. They select 3 sites in the Gambia but do not clarify their proximity to each other and their heartwater endemic history. That would be of value if that data is available. In addition, one location seems to have a high tick challenge whereas the other two were of lower challenge. The authors’ conclusion that serology and pCS20 PCR assay combined defines the heartwater status of a population is only correct if serology is specific for heartwater. There is compelling evidence that the MAP-1B ELISA detects antibodies in animals where known vectors of heartwater do not exist. Hence, this conclusion needs to be modified to put more emphasis on detection of the organism supported by biological data of infection through transmission or clinical evidence. It is unfortunate that tick infection acquisition followed by transmission experiments were not conducted on the neonates which would have provided the confirmatory evidence to conclude and confirm the KOCH’s postulates.

1. There are some areas of the data that are extrapolated to conclude the infection status of these lambs and kids which are not reliable. In particular, the use of serology and the indirect MAP 1B ELISA data to conclude that exposure to heartwater- E. ruminantium infection is present based on sero-positive results. Despite the fact that the assay has been shown to have high sensitivity for detection of anti-E.ruminantium antibody responses in sheep, it is the specificity of the assay that is of concern. Although it is documented that this assay is the most specific assay for detection of antibodies to E. ruminantium infection, there are numerous papers which demonstrate that false positive reactions are still detected and these could be due to an unknown agent that cross-reacts with E. ruminantium. The level of false +ve is variable from region to region and there is no data that defines this. At best one can only state that the sero-positive reactions in the presence of the tick vector Amblyomma variegatum or another vector species of this genus may be heartwater related. Since the animals were kept in an extensive farming system, their exposure to other
serologically cross-reacting agents can not be discounted. Hence the conclusions based on the serology should be TONED DOWN. In particular in the Introduction and on page 5 of M+M under Study sites lines 106-107. Emphasis on PCR+ve and clinical signs, postmortem brain smears should be made to state with certainty that the infection is due to heartwater/E. ruminantium infection.

Another important aspect to remember is that it is widely believed that antibody is not protective against heartwater and proposing the sero-positive state (soon after birth) as the resistant state and the sero-reversion state (older age), resulting in increased susceptibility should also be toned down. The elements in colostrum that are protective have not been defined in any target species. Nevertheless, there is interesting data from the serology, especially the sero-reversion after the initial positive state which at best represents a typical physiological phenomenon of transfer and decaying of passive antibody.

2. The pCS20 probe is in fact of low sensitivity for detection of carrier animals, but the pCS20 PCR assay has a higher sensitivity and can detect carrier animals. This needs to be corrected in line 81+82.

Line 89-91: The MAP-1B ELISA’s increased sensitivity to detect antibodies that react with E. ruminantium MAP1 in sheep or goats is not an inherent property of the assay but due to the fact that the kinetics of development of antibody responses in sheep and goats to E. ruminantium is different from that in cattle where sero-reversion occurs. Sheep and goats respond to heartwater infections by developing a prolonged antibody response, which is unique. This should be clarified in the text.

3. The Abstract and the text refer to 76 new born animals being monitored from birth to day 162. However, the data set shown in the Tables 2a and 2b add up to 62. (36 lambs and 26 kids). In Section M+M under Animals lines 110+111 the animals total 77. This issue is very distracting as the percentages and proportions are being described I believe on the total being 76 whereas the data set only shows 62. The authors should clarify. If this is an error it is inexcusable.

In addition, the data presented as lambs and kids separately is also confusing due to the fact that it obscures the comparison of the selected three sites especially since they seem to have a difference in tick attack rates. Hence, I strongly advise that the data be presented for each of the three sites separately and that in fact it could make a very nice comparison of the kids and lambs that exist at the sites Keneba and Bansang. This would also allow for the mortality data comparison per site and animal species in the same Table. A better correlation of PCR +ve animals, survival or mortality and tick infestation data may shed light on whether the in-utero infection has an effect on the fate of the infection after the neonatal resistance expires. Did the authors detect E. ruminantium by brain smear on brain samples of the animals that died during the study? They indicate that they did PCR on the brain. The gold standard of heartwater diagnosis is a positive brain smear. I invite a comment from the authors regarding this comment. PCR detects the organism DNA but these could be dead or alive…the brain smear would demonstrate the infection in the brain capillaries.

4. M+M Line 117-121. The authors state that they collected blood +/- EDTA on the day the animals were born or within 3 days of birth. Since they attempt to make a distinction between in-utero infection or via colostrum, they should show the data in that manner to demonstrate how many animals actually were PCR+ve at birth and how many were +ve within 3 days which would be either an in-utero route of infection or via colostrum. Interestingly the authors show no serology data for 9 kids for level 1, they should explain why this was the case?? Also they should separate the serology data for those at birth and within 3 days of birth, they show level 1 which is day 0-10 after birth. The suggested data presentation should be improved to make more definitive statements.

5. Did the authors validate their nested PCR assay in any way?, if so they should indicate this or refer to other publications if published.

6. Line 203 insert the word nymph after 1.

7. According to my calculations, there were 15 animals that remained negative by PCR not 19!!!

8. Line 210-211 the highest # of +ve animals were in level 5 days 78-98 not days 99-126. In fact during days 99-126 at least 50% or more of the lambs were not tested (nd I presume that it means “not done”!! There is no explanation for nd and why. This should be provided. Table 3: line 212: 6 of 9 were PCR +ve in Kaneba, these are not evident in the Table 3 and it should also be indicated in the text that Kaneba is where the tick attack rate was highest/severest and that has an implication that in higher challenge there will be more transmission to neonates, from mother to neonate. What happened to the 21 kids at Kaneba, what was there fate??
9. Line 213: the sentence is ambiguous and refers to the MAP 1B ELISA “perform satisfactorily” What does this actually mean, each endemic or non-endemic site is different???

10. Line 223: The authors should indicate the reason for animal 2317 & 2318 “nd” for levels 1-3 serology –how do they know they were +ve during these times in context of what they present here?

CONCLUSION AND RECOMMENDATION: This paper has some very interesting data on heartwater epidemiology in lambs and kids from birth to day 162. However, what their objectives were, were not met. See first paragraph for details. The paper’s data is not well organized; the numbers of animals do not tally. Hence the conclusions are questionable. The data presented has the potential of being a good account of infection dynamics in neonate sheep and goats. This paper needs major revision in relation to the above mentioned criticisms before it can be accepted for publication. The authors should respond by making the suggested changes and re-submit after the major revisions, for re-evaluation changing their objectives and pointing out their findings to these objectives.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)