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: 1 Date: 19 February 2007

Reviewer: Eduardo Berriatua

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General

I understand the study aims at investigating the time from birth to 5-6 months of age, when small ruminants raised in the traditional rearing system of Gambia, become infected with Ehrlichia ruminantium, and elucidate whether infections at an early age, which are critical for E. ruminantium endemic stability, depend on vertical and/or tick-borne transmission. The infection state of animals is assessed by PCR and ELISA and results obtained with these techniques are compared.

The questions addressed in the study are important to improve our understanding of the epidemiology of Heartwater in small ruminants which is a major disease in sub-Saharan Africa. The article merits publication however, it needs substantial revision and include a better description of particular study aspects, reorganise some of the information, perform a more complete statistical analysis and improve interpretation of some results.

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

Introduction

The introduction to the study is adequate, but the following needs addressing:

• Between lines 89-91 authors acknowledge problems in the past for specific E. ruminantium serological diagnosis and subsequently imply that this has been overcome with MAP1-B ELISA having greater sensitivity (Se) than older tests. Has this ELISA also improved diagnostic specificity (Sp)?

• Last sentence: I would not say that the objective of the study is to “investigating the role of tick transmission in initiating an endemically stable state in indigenous small ruminant exposed to field tick challenge”, because to this you should have monitored tick infection more frequently in a greater number of animals. I would say that your aims were to monitor E. ruminantium infection and provide some information to better understand the origin of infection.

Methods

• It is essential that you give a better description of the traditional extensive rearing system of small ruminants in The Gambia, particularly with regards to place of rearing during the study (from birth to 162 days old), whether indoor or outdoor as this conditions tick exposure, and other aspects such weaning age, feeding habits, type of grazing ground, characteristics of livestock enclosures...

• Lines 110-111. You say study animals comprised 29 lambs..21 kids…13 lambs…10 kids and 4 lambs…This makes 77 animals not 76 as you say before (line 102).

• How long before had the dams at Kerr Seringe been treated with acaricidals for? Could this have prevented or influenced tick and E. ruminantium infection in dams and its vertical transmission to newborn animals?. You need to address.

• Were blood samples from newborns always taken after they had ingested colostrum? This is important to interpret PCR and serological results.
You must give more detail regarding age at first sampling. Saying it was done within 3 days of birth is not quite enough in order to interpret PCR findings because strictly, in 3 days ticks can infect, feed and drop off. Did you check for tick infection before taking blood samples? If you convince the reader that newborns were not exposed to ticks during the first 3 days of life and that your PCR is highly specific, then PCR-positive results strongly suggest vertical transmission of E. ruminantium as observed in cattle and this would be a major finding of your study.

Hence, next question is: What is the PCRs Se and Sp? What is the ELISAs Se? Move details on ELISAs Sp presently in the results section (page 9, lines 213-15) to materials and methods.

You need a separate section for epidemiological and statistical analysis (lines 172-179), which is now included under “Indirect MAP-1 ELISA”

Comparing ELISA and PCR results using Spearman’s rank correlation is insufficient. I suggest you complete this analysis for example by estimating the Kappa coefficient and degree of agreement between tests.

Results

The number of animals in tables 2a and 2b adds up to 62 animals (36 lambs and 26 kids), not 76 or 77 animals as you mention earlier. Are you excluding results from the dead ones? If so, why?

You say the highest number of PCR-positives was at 99-126 days but in table 2a most animals this age were not tested – please explain.

You say almost 19% of animals were PCR-positive when tested shortly after birth and using tables 2a and 2b, the estimate would be almost 18%(11/62).

Did animals that died of Heartwater have clinical signs prior to death and lesions on post mortem examination compatible with the disease? Is the presence of E. ruminantium in tissue alone a definite diagnosis of the cause of the death?

Discussion

Discussion is generally comprehensive and it could be improved by adding interpretations to some of the questions that I raise above. Other things are:

Line 274. Specify the test to which animals tested negative and mention here the possibility that some PCR-positives may have been false positives if the PCR test is not 100% specific.

Line 279. I wouldn’t use “Infection rate” as a synonym for seroprevalence, it would be more appropriate to start with something on the lines of : “Prevalence of infection with E. ruminantium during the study estimated by MAP1-B ELISA, ranged 10-90%.

Results

Apart from previous comments, I find Tables 2a, 2b and 3 very informative, and could be further improved by indicating animal’s location. Moreover, you say in lines 218-19 that these tables “compare” ELISA and PCR tests and they do not, they merely “describe” results. If you wish them to “compare” you could for example add an extra column with kappa coefficients or other alternative comparison statistic.
is evident that after a PCR-positive results animals were often PCR-negative on subsequent samplings. What was the probability for having a repeated PCR-positive result after being PCR-positive a first time? This is useful information for understanding the pathogenicity of infection the validity of the PCR test.

Discussion

• Lines 281-85. It is sensible to assume that observed seroprevalence time pattern is the results of maternally derived antibodies. My comment is that it would have been wise to test the dams by ELISA and PCR in the perinatal period as this would have allowed you to correlate dam and offspring results and probably support some of your conclusions. Maybe you would like to comment on this.

• Line 292: I would appreciate a brief explanation for “a down-regulatory effect on the production of antibodies”.

• Lines 295-298. Interesting finding indeed. What about newborns being imunotolerant to the infecting E. ruminantium strain and not developing an antibody response? This occurs in ruminants following pestiviral infection (Bovine Viral Diarrhoea and Border Disease) in utero during early pregnancy. Maybe worth mentioning this.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.