Reviewer's report

**Title:** Cryptic Leishmania infantum infection in Italian HIV infected patients

**Version:** 4  **Date:** 3 April 2009

**Reviewer:** Joaquina Martin-Sanchez

**Reviewer's report:**

**Major Compulsory Revisions**

The differentiation between limit of detection and limit of quantification is not based on valid criteria. The non-reproducibility at 100% of the positivity, when the quantity of DNA is very small, is simply attributable to a law of probabilities. In consequence, I will not be able to consider the manuscript acceptable for publication if the authors do not use 0.01 parasites as a positivity threshold. If the authors claim (as they have done with other modifications suggested in the reviews) that the number of positive samples does not change, I will have to conclude that they are pulling my leg.

The authors should find a statistician specialised in regression analysis to help them perform the tests and interpret the results. For example, in the multivariate analysis we observe how the independent variable ‘CD4 cell count’ loses its significance (p=0.386), therefore this variable must be removed from the model, which will therefore be reduced to one with the independent variable ‘Viral load’. This means that the authors cannot continue to retain the comments regarding the association between the parasitic load and CD4 cell count in the results and discussion, since this association does not exist. Given how clear it seems that the authors are lacking in sufficient knowledge in this field, I have serious doubts as to whether they have checked that the variables meet the necessary conditions to apply a lineal regression, for example the lineal relationship between the variable dependent and each of the independent ones. I also have serious doubts as to whether the authors have checked if there is interaction between the two independent variables analyzed (which is highly probable), and if they have done so, they should make some comment on the matter.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests