Review by anonymous reviewer, 12 Jul 2022 15:36

In this preprint written by Peylhard et al., whole blood transcriptome profiles of five cattle breeds infected by Trypanosoma congolense were compared in order to find molecular differences between resistant and non-resistant breeds to trypanosomosis. Authors observed transcriptomic changes before and after infection in all breeds. Differentially expressed genes were also detected between tolerant and susceptible breeds and, based on these, the importance of different molecular functions for the trypanosomosis tolerance is discussed. The novelty of the work is, mainly, the studied breeds, since some of them had been overlooked until date. The introduction explains clearly the context of the study and it can be easily understanded by non-expert readers. Nevertheless, there are some aspects of the article that may be improved before submission:

1. I miss information about why was expected to find Trypanosoma congolense RNA in the bovine blood samples. Was this described previously? What mechanism introduces the RNA of the parasite into de host blood?
Reply: *Trypanosoma congolense* is an extracellular parasite that multiplies in the blood of its vertebrate host (line 54 of the article), so molecules of trypanosomes are found in the blood. In addition, molecular diagnostic tools use blood to detect DNA or RNA of the parasites.

2. Some figures may be improved. For instance, some labels in Figure 2 are unreadable, so it would be desirable to replace them by different shapes. Furthermore, the colors of Figure 4 should be more friendly.
Reply: As suggested, we modified Figure 2 to make it more readable. Different shapes correspond to the breeds, and different colours correspond to the treatment Breed*Time points. In addition, we used more common colours in Figure 4.

3. Differential expression analysis was performed through multiple pairwise comparisons. Given that this is a longitudinal experiment, I recommend exploring other methods designed for this kind of data that could return results overlooked by the pairwise comparisons approach.
Reply: We agree that there are actually several ways to analyse the data, and methods designed for longitudinal data could be tested. Nevertheless, with the presence of only 4 time points, we are a bit limited to have a good capture of temporal trends. We also think that performing pairwise comparisons as we did remains a relevant way to explore the kinetics of host response to infection in each breed and the basal differences between breeds, which allows to meet the main objectives of our study. This is why we chose to focus on the in-depth analysis of these results without including additional statistical analyses. Testing other methods will be made possible, knowing that all generated data will be freely available

4. Authors should clarify why an FDR threshold of 0.001 is adequate because 19 comparisons were performed. In addition, they should provide more details about the rationale of choosing different FDR thresholds for the functional analyses.
Reply: The choice of a p-value or a FDR is always arbitrary. In our case, since there are thousands of tests for each of the 19 contrasts, to declare a gene as differentially expressed, we chose a FDR of 0.001, based on a classical FDR of 0.05 divided by 19 contrasts (0.05/19=0.0026, value rounded to 0.001). Concerning functional analyses, the three functional categories of IPA (Diseases and functions, canonical pathways and upstream regulators) do not contain the same quantity of information and thus the number of tests

performed by IPA are different depending on the functional category. We tried to clarify this in the main text, at lines 226 and 248-249.

5. I recommend considering meta-analyses as the approach to compare the results between comparisons. Comparing the lists of genes that pass arbitrary thresholds may lead to miss relevant information.
Reply: We thank you for this suggestion. In order to present a first in-depth analysis of our transcriptomic data that responds to our main objectives and to avoid making the text more cumbersome, we prefer to focus on the analyses we first proposed in a classical framework. Meta-analyses could be performed as an independent study reusing our data, which will be freely available, to respond to other specific questions.

Review by anonymous reviewer, 22 Sep 2022 11:14

The manuscript entitled "Whole blood transcriptome profiles of trypanotolerant and trypanosusceptible cattle highlight a differential modulation of metabolism and immune response during infection by Trypanosoma congolense" is a very comprehensive study which aimed at improving the knowledge of the biological processes involved in trypanotolerance versus trypanosusceptibility using experimental Trypanosoma congolense infections in cattle. To this end, a whole blood genome-wide transcriptome of three trypanotolerant taurine breeds (N'Dama, Lagune and Baoulé), one susceptible zebu (Zebu Fulani) and one African taurine x zebu admixed breed (Borgou) were profiled by RNA sequencing at four time points, one before and three during infection. Moreover, most work performed by others in the past only consists of a comparison between one trypanotolerant and one trypanosusceptible cattle breed or only focusses at one particular time point. Hence, the manuscript presented now consists of a more thorough and kinetic study which strengthens the relevance of the observations (even be it at the gene-level). Moreover, this study revealed previously overlooked features that might contribute to susceptibility/tolerance towards T. congolense infections such as a strong disturbance in host metabolism and cellular energy production that differentiates trypanotolerant and trypanosusceptible breeds.

Therefore, I believe this research provides a unique opportunity as well as the basis for better understanding of the biological mechanisms at work during African trypanosome infections, especially concerning the interplay between immunity and metabolism that seems differentially regulated depending on the cattle breeds. Below I give a brief yet detailed overview of the strength/weaknesses of the manuscript.

1. Title

The title clearly reflects the content of the article.

2. Abstract

The abstract is concise and presents the main findings of the study.

3. Introduction

The introduction gives a very good overview of how trypanosusceptibility and trypanotolerance is defined as well as the current status regarding technologies used to understand trypanotolerance. To this end, relevant old/newer articles were sited. Moreover, the authors also provide a very good rational for using besides the well-characterized trypanotolerant N'Dama breed, also two overlooked trypanotolerant breeds, Baoulé and Lagune, the Borgou crossbred breed, and one trypanosusceptible breed, the Zebu Fulani. Hence, this would strengthening the relevance of the study! Furthermore, the authors clearly define what the study will focus on; i) breed-specific transcriptomic signatures in blood before infection, ii) main genes and biological functions that responded to infection, whatever the breed, iii) breed-specific transcriptomic profiles during infection, and iv) basal and dynamic transcriptomic profiles that could be associated with trypanotolerance.

4. Materials and methods

This section contains detailed information with respect to the technologies used and the strategies used to analyze the massive amount of data. Consequently, this should be sufficient to allow replication by other researchers. Regarding the statistical analysis, I am not an expert in this aspect but according to me (based on many articles using the same technology) it seems that the statistical analyses are appropriate.

5. Results

All results are presented in a concise manner and the message the authors want to transmit is clear. The fact that different breeds of trypanotolerant animals are used and a kinetic study was performed further strengthens the relevance of the results. I am not an expert in statistical analyse and therefore cannot comment on this aspect.

Given the fact that (i) several of the authors are experts in the field and have a very good reputation and (ii) I am familiar with some of the work of the authors, I do not suspect scientific misconduct.

6. Tables and figures

All the figures and tables (including the supplementary figures/tables) are understandable without reference to the main body of the article. **In addition, figures and tables have a proper caption.**

7. Discussion

Though the discussion section is very elaborate it is well structured in subsections and discussed accordingly. Also, the discussion allows concluding that a T. congolense trypanosome infection has a major impact on the cattle blood transcriptome, whatever the breed. As pointed our correctly by the authors, this research provides a global transcriptomic picture of infection as well as during infection. Interestingly, the results obtained by others are confirmed in this very elaborate study such as a strong regulation of the immune system functions with an early activation of innate immune response, followed by an activation of humoral response and an inhibition of T cell functions at the chronic stage of infection. The fact this change in the transcriptome (i.e. DEGs, functions and biological processes) is observed in all breeds, yet there are differences in gene expression dynamics in these three trypanotolerant breeds suggests that AFT breeds, although subjected to the same selective

pressure caused by trypanosomes, may have developed different adaptation mechanisms. This is again a very important conclusion that has been suggested by many others and further strengthens the importance of the work of the authors!

I also appreciated that the authors mentioned that "It would be worth exploring other African zebu breeds to confirm if this observation is a global feature of indicine breeds", which according to me would be very important to investigate.

Finally, the fact that the authors also referred to work performed in murine models of African trypanosomes and discuss similarities/discrepancies is also appreciated.

The only minor point of criticism I might have is that it is important to mention that this research is focused on T. congolense infected cattle, which is the major cause of Nagana within the African continent.

The authors mention at the end of the discussion that "this could pave the way to further refine the interactions between immune response and metabolism in cattle which in turn could improve preventive and curative measures of AAT and also other infectious diseases".

Yet, cattle can also become infected with T. brucei brucei or T. vivax which can also cause AAT and within these infections, the genes/pathways affected might be different and the underlying mechanism involved in pathology (anemia, liver injury,;..) could be different. Therefore it would be very important to perform a similar study on these trypanosome infections before drawing general conclusions about AAT.

Reply: We thank you for your positive comments on our study. We took them into account by adding a sentence at lines 827-828 to suggest additional work using *T. brucei brucei* and *T. vivax*, and specifying "due to *T congolense*" at lines 803 and 827 to remind that our experiment model used this species.

8. References
The authors used appropriate references to confirm/justify/explain their results and also refer to other relevant research that confirms/contradicts their work.

Overall this is a very well-written and impressive research paper that provides new and valuable data to contribute to a better knowledge of African livestock genomics and to decipher the pathogenic process in bovine trypanosomos