Reviewer's report

Title: Co-infection of human parvovirus B19 with Plasmodium falciparum contributes to malaria disease severity in Gabonese patients

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Reviewer: Svetoslav N Slavov

Reviewer's report:

Human parvovirus B19 (B19V) is an ubiquitous virus throughout the human population and its clinical impact depends on the clinical and immunological status on the infected hosts. In this relation, studies examining the co-infection between B19V and the malarial plasmodium are of particular importance, where both virus and parasite could influence the outcome of the severe anemic state. In this aspect the manuscript brings significant positive value, examining the DNA prevalence and the outcome of the co-infection Plasmodium spp./B19V in children from Gabon. It also brings very interesting genotyping data, demonstrating the B19V genotype 1B in Gabon, which is considered an “Asian” genotype because of its characterization in Vietnam. The characterization of B19V genotype 2 is also of significant value, due to recent discussions that it is disappearing from circulation in the Northern hemisphere and is being substituted by the genotype 1B, which has very extensive worldwide distribution. I was surprised that genotype 3 was not detected among children in Gabon, once it is with typical African endemicity. However, regional circulations of B19V in the African continent with its extensive territory, could have influenced the spread of B19V genotype 3 in Gabon. However, the manuscript suffers from some shortcomings which I suggest to be reviewed by the authors.

1. The scientific English should be revised by a native speaker. I strongly advise a correction due to the extensive use of words, the incorrect use of articles, the inadequate conjunction of verbs, where conjunction is necessary etc. This only will bring a positive value to the manuscript and clarity to the reader.

2. Background:

2.1. The word “poverty” in the beginning is not used rightly. I suppose that the authors meant “poor”, but nevertheless, this is a very strong word that cannot be used in a scientific context. Malaria was a widely spread disease, even until recently, when large areas of the Southern Europe, South America, Central and Southeast Asia, were devastated by continuous epidemics and foci of the disease. I suggest to use only the words “undeveloped countries”. Moreover, the initial phrase is not very clear, and I suggest to clarified or split into two phrases.

2.2. The genus of B19V is Erythrovirus and not Erythroviruses.

2.3. It could be mentioned that B19V causes, a wide spectrum of clinical diseases, but that they depend on the hematological and the immunological status of the host. In this relation the authors, should clarify which are the
conditions that B19V induces in healthy subjects and which are induced in patients with altered immunity and hematopoiesis.

2.4. I did not understand is there any relationship between B19V and malaria in the study from Niger. If, yes please specify in the sentence. For example “in severe anemia induced by Plasmodium spp.” etc.

2.5. …in a cohort of P. falciparum infected patients. Please specify in the sentence

3. Methods

3.1. In what type of hospital was performed this study? Was it a field study or not, what type of villages it included: tribal or not. This should be clearly mentioned in the Methods section. Moreover, a brief geographic characteristic of the examined region should be provided as the majority of the readers probably do not know where is Lambarene.

3.2. Substitute “pupils” by children or infants.

3.3. Substitute the “matching partners” by control individuals.

3.4. There is no information in the materials section how the B19V serology was performed. Please, include section named “B19V serology evaluation” or similar. In this section, please include the kits, and the methodologies, which were used for the evaluation of the titer of anti-B19V IgM and IgG.

3.5. What was the sensitivity of the real-time PCR, when the international standard for B19V was applied (in IU) and how it was compared and extrapolated to genome equivalents. If a formula was used, should be provided. Give a detailed explanation in the further correspondence.

3.6. Were the obtained sequences submitted to GenBank? Please provide the numbers of the submissions, because they will be used for further analyses, especially for subgenotype 1B.

3.7. What the authors mean by the “Obtained sequences were matched with the NCBI GenBank……” and cite the access number of the B19V strain Au once the authors had genotype 2 and subgenotype 1B. The Au strain is not a reference for the above mentioned genotypes. Please, make sure the adequacy of the prease.

3.8. For the sequencing analysis, was used very short fragment although amplifying one relatively variable region and Big Dye is a registered mark of the Life Technologies, USA and not Perkin Elmer. The primers for sequencing although already designed must be given in the manuscript and cited.

3.9. For the phylogenetic analysis it was used very limited number of sequences. Why the authors align the sequences using BLAST? The algorithm for the alignment which they used belongs to what software-BioEdit? How the authors confirm the reliability of the constructed alignment by BioEdit?? What is the test from the BioEdit for the reliability test? The alignment must be checked for significance using the permutation probability test and this significance must be outlined in the next version of the manuscript with the value of P. In the phylogenetic analysis must be performed with more reference strains and the bootstrap probabilities must be compulsory shown on the final cladogram? How
we could evaluate the significance of the tree without the bootstrap significance on its branches?? The isolates on the tree must be represented by their original names and not by their GenBank accession numbers. The subdivision of genotype 3 is not well demonstrated on the performed analysis.

4. The first paragraph of the results section repeats the materials section, where these subdivisions of the groups are described. Please, exclude the unnecessary information because it is very repetitive.

4.1. It is not prevalence of B19V genomes but prevalence of B19V DNA or B19V viremia. Please correct in the text. Why the authors examine the seroprevalence only in the positive patients. I think that for the next version of the manuscript should be presented the overall seroprevalence of anti-B19V IgG in the entire group and not only in the positive patients.

4.2. How the authors confirm that the detected viral load is from acute infection and not from a persistent one? B19V establishes frequent persistency and could persist at the detected values for very long periods of time. This B19V DNA coincided with the production of IgM?

4.3. B19V/Plasmodium spp. is not a parasitemia. It is a co-infection between B19V and the Plasmodium spp. Please correct in the text.

4.4. Please calculate the genetic distances among the detected genotypes and put them into the text in the section of genotyping.

5. The authors should have in consideration some facts during the interpretation of their results. B19V infects the progenitor cells in bone marrow and this is not a target place for the Plasmodium spp. Please make a detailed revision of the obtained results in comparison with other findings in the field. Take care when involving genotype 2 in pathogenesis of malaria, because it can reflect simply B19V epidemiology in the region and not at disease outcome of the malaria.

5.1 What is HI-virus? Did the authors mean HIV? Please do not use “our patients” and the patients with malaria etc. The patients are not yours.

Level of interest: An article of importance in its field

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.