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Journal Name:	**South Asian Journal of Parasitology**
Manuscript Number:	**Ms_SAJP_82743**
Title of the Manuscript:	**Complement-mediated killing of Leishmania martiniquensis promastigotes**
Type of the Article	**Original Research Article**

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments		
Minor REVISION comments	The work seems to be well conducted and certainly adds knowledge about this *Leishmania* species that has been described more recently. However, I have some comments and suggestions that aim to contribute to the improvement of the manuscript. I recommend a full review in English In the summary: The sentence "These preliminary data may provide a further study for the explanation of the anatomical sequestration or immune evasion strategy of *Leishmania* species". It looks a little vague and this subject should be better explained. In Materials and Methods: The number of blood donors and the characteristics of each must be included. Such as age, sex, etc. The lethal effect on *L. martiniquensis* promastigotes was determined at a ratio of 1:1 (volume by volume) for 15, 30, and 60 min. I would suggest adding higher sera dilutions to the experiments. Question: In the Mat Met it was described that the lethal effect on *L. martiniquensis* promastigotes was determined at a ratio of 1:1 (volume by volume) for 15, 30, and 60 min. Nonetheless, in the results it was mentioned that the lethal effect of sera became undetectable by diluting the serum beyond 1:16. I suggest adding a table showing the results with the different dilutions of sera used in the experiments. Results: Considering that supposedly different serum dilutions were used in the experiments, it must be specified in the legend of Figure 1 which dilution corresponds to the results. In figure 1, referring to the results corresponding respectively to Fresh NHS* 30 and 60 min, as well as to 56°C 30' treated and 50°C 15' treated at 15, 30 and 60 min. Based on the variance of the results showed in the graphic its quite probable that the differences they are not significant. Discussion: On what was discussed by the authors: It is important to highlight that despite the complement action, those trypanosomatids that infect mammals, including man, have been co-evolving with their hosts for millions of years and, consequently, developing different escape mechanisms to survive and promote infection. Thus, as usual, factors related to variability for both parasites and hosts always interfere with the fate of the infection. Even in the results presented in this manuscript, it is clearly shown that a percentage of the parasites can survive and consequently they could be able to infect the host under natural conditions. Taking into account the following points presented in the discussion: A) "Investigation for MBL of the surface demonstrated positive using the immunofluorescence test. MBL is a well-known complement activator and an efficient opsonin. Binding and promoting lysis of live promastigotes of *L. braziliensis* by MBL were reported [16]." B) "Results of our study show that the biological circumstance of the parasiticidal	

	action in normal serum requires further study. It is tempting to theorize that this phenomenon may play a role in the natural host defense against this parasite". C) " An alternative complement pathway is seen as the major essential pathway that can eliminate various species of the *Leishmania* pathogen [13] D) "Serum MBL binding of *L. braziliensis* promastigotes also suggested the possible contribution of the lectin pathway in the pathogen killing [16]. MBL is a calcium-dependent plasma lectin that can bind to various microorganisms. It has shown a strong association with the severity of microbial infections [12, 26]. The binding of MBL by the pathogen could subsequently spur complement activation by increasing uptake of PMN (opsonophagocytosis) [11]". It is quite important to remember that: "During the initial interaction between parasites and macrophages, different species of *Leishmania* are recognized by a variety of macrophage receptors, including complement (CRs), Fcg (FcRs), fibronectin, and mannose receptors (MR). The recognition of the parasite by different receptors may impact the fate of intracellular parasites as well as the course of infection. Therefore, it is highly likely that, during natural infection, *Leishmania* are recognized simultaneously by more than one host cell receptor, and that specific combinations of these receptors result in differential activation that distinctively contributes to intracellular parasite survival. The recognition of *Leishmania* parasites mainly via CR3 and CR1 inhibits inflammation and oxidative bursting, in addition to leading to the accumulation of LAMP1 and Cathepsin D in parasitophorous vacuoles (PVs). A study investigating CR3 recognition found that this receptor was associated with the uptake of metacyclic parasites, a more infective form of *Leishmania*. These authors also found that the mannose receptor, in combination with CR3, is associated with the uptake of avirulent promastigotes. Another study found that the presence of the CR3 cluster in caveolin and cholesterol containing microdomains leads to delayed lysosome fusion, thusly favoring the replication of parasites within PVs. Together, these data show that *Leishmania* uptake via CR3 recognition could support the intracellular survival of these parasite species. **On the other hand**, the activation of complement receptors together with fibronectin receptors was shown to lead to an inflammatory response, thereby reducing parasite survival. It was also demonstrated that *Leishmania* parasites degrade fibronectin in a GP63-dependent manner. The uptake of parasites via mannose receptor recognition may also trigger an inflammatory response by host cells, as well as provide more efficient delivery of hydrolytic enzymes into the macrophage phagolysosome. On the other hand, FcgR mediated phagocytosis in bone marrow-derived macrophages (BMDM) was shown to promote IL-10 expression, which favors parasite survival and replication". (doi: 10.3389/fimmu.2019.02523).	
Optional/General comments		

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PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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