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Letter to the Editor

Response Comment on "*Detection of Lophomonas blattarum* (Order: Hypermastigida from Iranian Patients with Allergic Rhinitis"

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Dear Editor –in-Chief

Regarding the questions about our new article" Detection of *Lophomonas blattarum* (Order: Hypermastigida from Iranian Patients with Allergic Rhinitis" published in the Iran J Parasitol: Vol. 17, No. 4, Oct-Dec 2022, pp.583-588, I write this passage to clarify many facts about *Lophomonas*.

First of all, in our scientific world, one of the essential aspects of a reliable method is reproducibility. To accept a method, it should be reproduced in the same condition. The author of this letter referenced a paper on the molecular detection of *Lophomonas* in 2019 (1). While after four years have passed there is no similar study proving it all over the world. All the subsequent molecular papers are just related to the same author who did it in 2019 (2-4).

In our laboratory, we tried to amplify the small subunit ribosomal RNA (SSUrRNA) gene using the primers designed by Fakhar et al. Although the band obtained from the positive DNA samples did not agree with the band introduced by them, many changes were ob-

served in the sequence of those samples, which did not confirm the results of this researcher. Many colleagues from other countries also have tried to use the mentioned primers referenced by Fakhar et al in 2019, but unfortunately, they could not be reproducible. As a result, we cannot reference this article for molecular detection of *Lophomonas* until approval of this PCR method. Beside, after 2019, many articles reported *L. blattarum* only by microscopic diagnosis and all of them published in many scientific journals without any implementation of PCR methods, which probably showed they also could not apply that PCR method (5,6,7). According to these facts, in the present time, the diagnosis of *L. blattarum* in human samples was based on the morphological characteristics of the protozoon observed in respiratory secretions under optical microscopy (5,6,7). We believe that because of an undocumented article at the present time, we cannot change our gold standard for diagnosing *Lophomonas*.

Second, *Lophomonas* has two species (*L.blattarum* and *L.striata*) that live in the insects' gut. "While *L. striata* has no importance in public health, *L.blattarum* is the causal agent of lophomoniasis, a respiratory tract parasitic infection that mostly affects children and immunocompromised adults" (8). As mentioned before because of the lack of a reliable PCR method for the detection of *Lophomonas*, we are unable to distinguish *Lophomonas* by molecular method. In addition, there are several references reporting *L.blattarum* as a causal agent of respiratory infections (5, 8).

Third, in our project, all the samples were examined for the presence of *Lophomonas* by expert parasitology staff and all the aspects of the microscopic detection method were implemented for an exact diagnosis. Confusingly, the reference mentioned in the third question did not represent any document on the density of the parasite or severity of infection (2). Surfing the scientific websites showed there is only one article about the severity and density of *Lophomonas* infection which represents this issue " Based on our experience in the INRCL, an innovative severity index (SI) for *Lophomonas* infection was scored as mild to severe parasite density by counting parasites per high-power microscopic fields (HPF) (400×). Accordingly, mild density was defined as 1–10 parasites/100 HPF, moderate as 1–10 parasites/10 HPF, and 1–10 parasites/HPF were considered as severe" (9). Besides that, this method is an innovative method for the severity detection of *Lophomonas*, we believe that this method could not be an appropriate way for use in the laboratory as a standard method because samples for searching *Lophomonas* are mucoid and must be homogenized, and for this purpose, samples must be centrifuged which can affect the motility of *Lophomonas* and the detection of this protozoan can be confusing.

In conclusion, there is no standard method to measure *Lophomonas* load infection at present. We believe that until the invention of an appropriate method for the detection of *Lophomonas* load, the presence of this motile and alive multi-flagellated protozoan could be the best key to diagnosing all the samples.

However, our team is working on designing new primers and amplifying the other genes to diagnose this protozoan correctly. The gold standard of *Lophomonas* diagnosis is microscopic, using direct smears or stained ones. By the time of achieving a reliable method of PCR, we believe that the best way of detection *Lophomonas* is based on a microscopic procedure (7, 8,10).

Conflict of Interest

The authors declare that there is no conflict of interests.

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