

Response to comments on article “Molecular characterization and phylogenetic analysis of *Microsporidia* and *Cryptosporidium* spp. in patients with multiple bowel biopsies from Fars Province, Iran”

Iraj Mohammadpour¹, Farzaneh Bozorg-Ghalati², Mohammad Hossein Motazedian^{1,3}

¹Department of Medical Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Zand Blvd, Shiraz-7134845794, Iran

²Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Velenjak Street, Tehran-1956944413, Iran

³Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Zand Blvd, Shiraz-7134845794, Iran

Corresponding Author: Mohammad Hossein Motazedian; e-mail: motazedm@sums.ac.ir

Dear Editor,

We read with immense attention and special penchant the letter by Agholi et al. entitled “Comments on article: Molecular characterization and phylogenetic analysis of *Microsporidia* and *Cryptosporidium* spp. in patients with multiple bowel biopsies from Fars Province, Iran” published in the *Annals of Parasitology* 2017, 63(1), 73–74 [1]. In response to this letter, we stress that the significant aims of our study were to “emphasize the importance of identification of *Microsporidia* and *Cryptosporidium* sp. in patients with chronic diarrhea, and to characterize the phylogenetic relationship of these isolated pathogens in multiple bowel biopsy specimens from immunocompetent patients referred to the Department of Pathology, Shiraz Medical School” (page 322) [2]. On the basis of our objectives, any of histopathological investigation, flow cytometry (CD₄ count), delayed typed hypersensitivity reactions (DTH) and so forth, were not in the scope of our goals. These topics are different research projects, which can be executed and published in various journals. In our research, a structured questionnaire was used to collect demographic, clinical and therapeutic data from all patients (page 322). The immunocompetent

condition of our patients were evaluated relatively based on laboratory diagnostic tests (CBC-Diff-Plt-ESR; complete chemistry tests; CRP-RF; and IgG-IgM-IgE-C₃-C₄ levels), which had been recorded in their dossiers. Plus, anybody of our patients had not been either solid organ transplant recipient or under therapy with immunosuppressive drugs (like Prednisolone, etc.).

In addition, only cryptosporidiosis (*C. parvum* genotype II) in our study was significantly associated with inflammatory reaction (ileitis). Inflammatory reaction (ileitis) was stated in our report (page 327) based on patient’s pathology report sheet. This is in agreement with previous studies, which *C. parvum* were associated with chronic diarrhea, vomiting, and inflammatory reactions in children [3,4]. Besides, Microsporidial DNA was observed in the jejunum of an immunocompetent patient with no significant pathologic change and a history of chronic diarrhea. This finding is in accordance with previous published articles and scientific textbooks [4–6]. Also, microsporidia can create latent and unapparent infections among immunocompetent individuals and are not just in AIDS patients [7–10]. This is in conformity of our paper and findings (Page 327).

Furthermore, Dr. Agholi exclusively prepared two diarrheal smears on glass slides from one of his HIV⁺/AIDS patients infected with *Cryptosporidium* sp. as positive control. We are very grateful and appreciative for his kindly presenting gift. Unfortunately, when the quantity of extracted DNA from these two slides was determined by measuring optical absorbance at 260 nm using a Nano spectrophotometer, no appropriate measure and quality was procured (A_{260}/A_{280} was less than 0.5). Moreover, nowise *E. bienewsi* as positive control was bestowed by Dr. Agholi and he was entirely attentive of this. Therefore, positive controls were supplied from elsewhere.

Additionally, when we search for similar sequences of the 18S rRNA gene of *E. bienewsi* infection in humans from Iran deposited in GenBank database, neither sequencing data, nor accession number was available in GenBank by Dr. Agholi et al. So, the articles published by Dr. Agholi et al. were missed and not cited in our article and we are regretful about this happening.

Finally, in the conclusion paragraph, we notified that “The possibility that infection with *E. bienewsi* and *C. parvum* is associated with symptomatic disease in patients with a competent immune system needs to be clarified in further studies” (page 327). On the foundation of these remarks, our discussion was written absolutely in line with our goals, and quite clearly supported our results.

References

- [1] Agholi M., Taghinezhad A. 2017. Comments on article “Molecular characterization and phylogenetic analysis of Microsporidia and *Cryptosporidium* spp. in patients with multiple bowel biopsies from Fars Province, Iran”. *Annals of Parasitology* 63: 73-74. doi:10.17420/ap6301.89
- [2] Mohammadpour I., Bozorg-Ghalati F., Motazedian M.H. 2016. Molecular characterization and phylogenetic analysis of Microsporidia and *Cryptosporidium* spp. in patients with multiple bowel biopsies from Fars Province, Iran. *Annals of Parasitology* 62: 321-330. doi:10.17420/ap6204.68
- [3] Cama V.A., Bern C., Roberts J., Cabrera L., Sterling C.R., Ortega Y., Gilman R.H., Xiao L. 2008. *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. *Emerging Infectious Diseases* 14: 1567-1574. doi:10.3201/eid1410.071273
- [4] Gutierrez Y. 2000. Diagnostic pathology of parasitic infections with clinical correlations. Oxford University Press, New York, USA.
- [5] Texier C., Vidau C., Vignes B., El Alaoui H., Delbac F. 2010. Microsporidia: a model for minimal parasite-host interactions. *Current Opinion in Microbiology* 13: 443-449. doi:10.1016/j.mib.2010.05.005
- [6] Fishman JA. 2010. Cytopathology of infectious and inflammatory diseases. In: *Diagnostic Pathology of Infectious Disease*. (Ed. R.L. Kradin). Saunders Elsevier, Philadelphia, PA, USA: 23-76.
- [7] Sak B., Kváč M., Kučerová Z., Květoňová D., Saková K. 2011. Latent microsporidial infection in immunocompetent individuals – a longitudinal study. *PLoS Neglected Tropical Diseases* 5: e1162. doi:10.1371/journal.pntd.0001162
- [8] Sak B., Brady D., Pelikanova M., Kventonova D., Rost M., Kostka M., Tolarova V., Huzova Z., Kvac M. 2011. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *Journal of Clinical Microbiology* 49: 1064-1070. doi:10.1128/JCM.01147-10
- [9] Abreu-Acosta N., Lorenzo-Morales J., Leal-Guio Y., Coronado-Álvarez N., Foronda P., Alcoba-Flores J., Izquierdo F., Batista-Díaz N., Del Águila C., Valladares B. 2005. *Enterocytozoon bienewsi* (microsporidia) in clinical samples from immunocompetent individuals in Tenerife, Canary Islands, Spain. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 99: 848-855. doi:10.1016/j.trstmh.2005.05.010
- [10] Didier E.S., Weiss L.M. 2011. Microsporidiosis: not just in AIDS patients. *Current Opinion in Infectious Diseases* 24: 490-495. doi:10.1097/QCO.0b013e32834aa152