Author’s response to reviews

Title: Hepatic paragonimiasis in a 15-month-old girl: a case report

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Author’s response to reviews:

Dear Editor:

Thank you for your comments and suggestions. We have clarified the authors’ contributions and removed some names from the author list. We have learned much from your and the reviewers’ comments, which are very insightful and constructive. After carefully studying the comments and your advice, we have made corresponding changes in the manuscript (rendered as boldface in the text). The responses to specific comments are given below.

RESPONSE TO REVIEWER'S COMMENTS

Chia-kwung Fan (Reviewer 1): Although this paper is very interesting reporting an infant suspected of Paragonimus infection based on ELISA results performed by Sichuan Province Center for Disease Control and Prevention, however, if authors may provide the details about the ELISA results/data e.g., what kind of diagnostic antigens you used, positive control, negative control and patient's OD value etc will be better for reviewer to exclude any possibility of false positive reaction. Moreover, why not to perform a PCR detection on the liver specimen to confirm this case was infected by Paragonimus that will be much convinced. Without these convinced data, this diagnosis is too speculative and subjective.

Response: Thank you for your careful comments. We have added details on the ELISA data:

“Serum evaluation was positive for P. westermani antibodies, based on enzyme-linked immunosorbent assay (ELISA; Paragonimus westermani IgG Antibody Test Kit, Shenzhen Combined Biotech Co., Ltd. Shenzhen, Guangdong Province, China.), which was kindly performed by the Sichuan Province Center for Disease Control and Prevention).” (page 4, lines 10-12)
Our institution (West China Second University Hospital, Sichuan University) only received the positive serological result for paragonimiasis, but we did not receive any details of the assay such as diagnostic antigens used, positive control, negative control, OD value, etc. from the Sichuan Province Center for Disease Control and Prevention, as they did not preserve the results in their database.

We apologize that we did not perform PCR detection on the liver specimen to confirm this case. One of the reasons is that, according to the American Society of Parasitologist (https://www.cdc.gov/dpdx/paragonimiasis/index.html), “Diagnosis of paragonimiasis is based on microscopic demonstration of eggs in stool or sputum, but these are not present until 2 to 3 months after infection. (Eggs are also occasionally encountered in effusion fluid or biopsy material.) Detection of eggs in sputum or feces of patients with paragonimiasis is often very difficult; therefore, serodiagnosis may be very helpful in confirming infections and for monitoring the results of individual chemotherapy. Enzyme immunoassay (EIA) test has been the standard test for paragonimiasis; it is highly sensitive for diagnosis with serum samples of 96% of patients with parasitologically confirmed P. westermani infection. Specificity was >99%; Most published literature deals with pulmonary paragonimiasis due to P. westermani although in some geographic areas other Paragonimus species cause similar or distinct clinical manifestations in human infections” [Slemenda SB, Maddison SE, Jong EC, Moore DD. Diagnosis of paragonimiasis by immunoblot. Am J Trop Med Hyg 1988;39:469-471]. Furthermore, the small piece of liver tissue obtained was used for routine biopsy (hematoxylin and eosin staining), and no excess liver tissue was left for PCR detection.

Many reviews discussed that eggs provide a definitive diagnosis. However, eggs can be hard to find. Consequently, immunologically based tests are regarded as more sensitive for diagnosis [Blair D. Paragonimiasis. Adv Exp Med Biol 2014;766:115-152]. ELISA is one of the most widely used tests today [Lee MK, Hong SJ, Kim HR. Seroprevalence of tissue invading parasitic infections diagnosed by ELISA in Korea. J Korean Med Sci. 2010;25:1272-1276]. Furthermore, Narain et al. reported that IgG ELISA test has 100% sensitivity and 91.3% specificity in diagnosing paragonimiasis [Narain K, Devi KR, Mahanta J. Development of enzyme-linked immunosorbent assay for serodiagnosis of human paragonimiasis. Indian J Med Res 2005;121:739-746]. Additionally, Intapan et al. reported that IgG4 subclass antibody detection by peptide-based ELISA has 100% sensitivity, 94.6% specificity, 96.2% accuracy, 100% positive predictive value, and 88.9% negative predictive value in diagnosing human paragonimiasis heterotrema [Intapan PM, Sanpool O, Janwan P, et al. Evaluation of IgG4 subclass antibody detection by peptide-based ELISA for the diagnosis of human paragonimiasis heterotrema. Korean J. Parasitol 2013;51:763-766].

Furthermore, Fei et al. reported two adults diagnosed with hepatic paragonimiasis by ELISA and pathological findings of numerous eosinophils and Charcot-Leyden crystals [Liu F, Zhang J, Lei C, Wei Y, Li B. Feasibility of laparoscopic major hepatectomy for hepatic paragonimiasis: two case reports. Medicine 2016;95:e4939]; the same procedure was used to diagnose pulmonary paragonimiasis mimicking tuberculous pleuritis [Luo J, Wang MY, Liu D, et al. Pulmonary paragonimiasis mimicking tuberculous pleuritis: a case report. Medicine Apr 2016;95]. Mukae et al. reported that in 13 patients, eggs were found in the sputum of 4 patients, who were serologically diagnosed as having pleuropulmonary paragonimiasis using dot ELISA method to

Thus, we are confident in our diagnosis of hepatic paragonimiasis in our patient.

Ricardo Oliveira (Reviewer 2): This is an original and relevant case report that calls attention to an atypical manifestation of the disease in a 15-month old girl. Although the text is well written and with an appropriate language style, the author might provide more information in order to improve the quality of the manuscript, as follows:

Comment 1:
* Please describe at the 'Case Presentation' section all other laboratory tests and the methods that were performed for the properly diagnosis.

Response 1: We have described the other laboratory tests in the Case Presentation section:

“Blood analysis showed the following results: white blood cell count, 26.5 per 109/L (neutrophils, 20.0%; lymphocytes, 52.0%; monocytes, 5.0%); eosinophilia, 20.0%; C-reactive protein (CRP), 137.0 mg/L; alanine aminotransferase, 66 U/L; albumin/globulin, 0.9. Chest X-ray and CT showed no specific changes. We did not observe any parasite eggs in the stool, sputum, or bronchoalveolar lavage.” (page 3, lines 23-27)

Comment 2:
* As paragonimiasis is typically a parasite of the lung, it should be reported whether any diagnostic procedure has been performed in the lung.

Response 2: We apologize for not adding the examinations with negative results in the original manuscript, which is very important in differential diagnosis. We have added those examinations in Case Presentation:“Chest X-ray and CT showed no specific changes. We did not observe any parasite eggs in the stool, sputum, or bronchoalveolar lavage” (page 3, lines 26 and 27)

Comment 3:
* Although the parasitological diagnosis (in the faeces or sputum) presents low sensitivity, it must be performed, and the results, as well as the methodological details, must be informed.

Response 3: Same as response above.

Comment 4:
* Pg 4, Ln 5: it is reported that Sichuan is endemic for paragonimiasis. Which other helminth infection have epidemiological relevance in this province? Is it important to perform a differential diagnosis to any of them? Are Schistosomiasis or Fascioliasis an issue?
Response 4: Other helminth infection has epidemiological relevance in Sichuan province neither Schistosomiasis nor Fascioliasis. Since 2004, an integrated strategy was developed to control the transmission of Schistosoma japonicum in China. No S. japonicum infection was identified in snails since 2007. By 2014, 88.9% of the endemic counties achieved the transmission interruption of schistosomiasis and transmission control of schistosomiasis was achieved in the whole Sichuan province in 2008[Liu Y, Zhong B, Wu ZS, Liang S, Qiu DC, Ma X. Interruption of schistosomiasis transmission in mountainous and hilly regions with an integrated strategy: a longitudinal case study in Sichuan, China. Infectious diseases of poverty. Apr 07 2017;6(1):79.]. Fascioliasis, one of the major veterinary parasitic diseases in ruminants, such as cattle, sheep, and goats, causes economic losses in animal husbandry. Few published articles are available on human fascioliasis in China, with only scattered cases reported long time ago[Ai L, Cai Y-C, Lu Y, Chen J-X, Chen S-H. Human Cases of Fascioliasis in Fujian Province, China. Korean Journal of Parasitology. Feb 2017;55(1):55-60.]. The first outbreak of human fascioliasis in China was in Binchuan (southwest China), in 2012.

It’s important to perform a differential diagnosis to them. The diagnosis based on pathobiology assay test and immunological detection technology.

Comment 5:

* Pg 4, ln 8: please provide more methodological information regarding the ELISA performed for the serological diagnosis of paragonimiasis.

Response 5: Same as response to reviewer 1.

Comment 6:

* What is the sensitivity and specificity of the ELISA?

Response 6: Same as response to reviewer 1.

Comment 7:

* Is Paragonimus westermani the main specie found in Sichuan province?

Comment 8:
* Pg 4, ln 12-16: please replace the reference with a more up-to-date one.

Response 8: The references have been replaced (page 4, lines 16 and 20).

Comment 9:
* Pg 5, ln 28 and pg 6, ln 1: considering that no parasitological confirmation was obtained and that ELISA was the only positive test observed in the reported case, it is not possible to conclude that ELISA is an adequate confirmatory method for HP. What would be the gold standard method?

Response 9: In this patient, the diagnosis of hepatic paragonimiasis was established based on epidemiological characteristics, findings of Charcot-Leyden crystals on liver lesion biopsy, positive ELISA, and effective praziquantel treatment. We have added further explanation above (response to reviewer1) that supports our diagnosis.

Comment 10:
* Figure 2: Apparently the image shown in figure 2 is not very well stained, and does not allow the discrimination between eosinophils and other leukocytes. I would suggest the authors to perform the Chromotrope 2R staining in order to allow a better identification of eosinophils. Moreover, the image does not seem to be the most representative image of the specimen.

Response 10: Another image better stained has been chosen by the pathologist. As the small piece of liver tissue was used for routine biopsy (hematoxylin and eosin staining), no tissue is available for chromotrope 2R staining.