Author's response to reviews

Title: Misdiagnosed murine typhus in a patient with multiple cerebral infarctions and hemorrhage: A case report

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Author's response to reviews: see over
Dear Editors:

Thank you giving us a chance again to revise our research article entitled “Misdiagnosed murine typhus in a patient with multiple cerebral infarctions and hemorrhage: A case report” for consideration of publication at your prestigious journal.

This article had been revised after full consideration of the comments of two experienced reviewers. The responses are follows.

Reviewer 1

1- Line 66: revise the sentence “when he severe headache, fever and dizziness associated with nausea developed” there is no verb

   The sentence has been revised.

2- Line 73: you can change the sentence "intracranial infection" by encephalitis

   Intracranial infection was changed to encephalitis.

3- Line 82: there is space between A and repeated MRI

   A space was inserted between A and repeated MRI.

4- Line 87: there is space between basis and of

   The space was inserted between basis and of.

5- Line 107: symptoms instead of synptoms

   Symptoms was changed to symptoms.

6- Line 117: I think the diagnosis is meningoencephalitis instead of encephalitis

   Encephalitis was changed to meningoencephalitis.

Reviewer 2

Background: Please, update the references regarding the prevalence of endemic typhus in China. Although, the review published by Fan et al. in 1987 was a state of the art paper at the time, it is obviously quite outdated and the current status needs to
Currently, detailed data on the epidemiology of typhus do not exist, although some localized outbreaks of typhus have been reported. The existing Chinese epidemiologic data shows that the prevalence of typhus fluctuates between 0.25/10,000 and 0.49/10,000.

Case presentation: Please, clarify and provide a table or chart of patient symptoms so reader can better understand the time line of events and symptoms. As written it is not very clear if this patient was seen first on April 10 and treated symptomatically w/o success, and then he was admitted to neurology on April 24. Then only on the third day after hospitalization a rickettsial disease was suspected and appropriate antibiotic therapy was started (17 days after onset?), and blood was tested for anti-rickettsial antibody 2 weeks after onset. If the latter is true, why was treatment not started earlier?

Table 2 shows the time line of the event and symptoms.

The diagnosis was not apparent initially. The patient was transferred from a local hospital on 24 April (the onset was 10 April). Three days after admission (27 April; 17 days after onset), a rickettsial disease was suspected and appropriate antibiotic therapy was started. Blood was tested for anti-rickettsial antibodies at the same time (28 April; 18 days after onset).

Lines 81-87: It is indicated that this patient had an eschar on his leg, and this was used a clue to start antibiotic therapy. However, an eschar is not a typical symptom for murine typhus, but would rather be indicative of a spotted fever group infection. Please, provide necessary comments in the discussion section (therapy would be the same even if which rickettsial etiology was incorrect).

I agree that an eschar is not a typical symptom for murine typhus. In our patient, however, the presence of an eschar led us to suspect the rickettsial infection. Based on the patient’s history, he was definitely bitten by an insect.
Lines 94-95: Please, clarify what antigens the antibody titers were detected to? It is stated that 4 different antigens were used for testing, but which ones had the antibody response detected?

**Serum IgG against** *R. typhi*, *R. conorii*, *O. tsutsugamushi*, and *A. phagocytophilum* were detected using the IgG IFA kit (Fuller Laboratories and Focus Diagnostics, Cypress, CA, USA), according to the manufacturer’s instructions. Goat anti-human immunoglobulin G (γ-chain specific) was purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL, USA).

Lines 98-99: Please, submit nucleotide sequence of the fragment amplified from the patient blood to GenBank and include the accession number in the manuscript. Even if it is identical (as claimed by the authors) to the reference sequence, it is critical to provide a sequence of this unique voucher sample for however many nucleotides were obtained. Please, provide the reference or primer sequences for the method used for PCR detection and amplification.

For article length, we did not provide the detailed test method. The detail examination method follow:

A whole blood sample in EDTA-K$^{++}$ was obtained from the patient and sent to the Zhejiang Province CDC to confirm the diagnosis of typhus. DNA extraction and PCR were performed in a standard PCR laboratory, and negative controls were included during each step. DNA extractions from whole blood were performed using a Universal Genomic DNA Extraction Kit (version 3.0; Takara Biotechnology Co., Dalian, China) according to the manufacturer’s instructions. A nested PCR was used to amplify the *R. typhi* groEL gene fragment (217bp). The primers for the first round of amplification were as follows: Gro1 (5′ -AAGAAGGHGTGATMAC-3’); and Gro2 (5′ -ACTTCMGTAGCACC-3′ ). The primers for the second round of amplification were as follows: SF1 (5′ -GATAGAAGAAAAGCAATGATG-3’ ); and SR2 (5′ -CAGCTATTTTGAGATTTAATTG-3’ ). Twenty microliters of the extracted DNAs were used as template DNA in a 50-µl reaction mixture. The primary PCR
procedure was as follows: 94°C for 4 min; and followed by 40 cycles of 94°C for 40 s, 45°C for 40 s, and 72°C for 40 s. Ten microliters of the product from the primary amplification were used for the nest amplification in a 50-µl reaction mixture. The amplification consisted of 40 cycles (30 s at 94°C, 30 s at 56°C, and 30 min at 72°C each). Gel-extracted PCR products with the expected size were sequenced using an Applied Biosystems 3730 DNA Analyzer (ShangHai Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

Table: Please, indicate the exact days when each test was performed so the timeline is clearly seen. Why were the results of testing with rickettsial antigens not included?

A new table depicting the timeline of events and symptoms has been included in table 2. The results of rickettsial antibody testing is shown in Table 1.