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CASE REPORT

Case Report: A Case of Visceral Leishmaniasis Misdiagnosed as Brucellosis

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Abstract: Visceral leishmaniasis (VL) is an infectious disease caused by protozoan parasites of the genus *Leishmania* and transmitted through the bites of infected female sandflies. Due to its nonspecific clinical presentation, VL is prone to misdiagnosis and underdiagnosis. Though rare, VL is endemic in regions of Africa, South America, Asia, and parts of Europe, including the Mediterranean. This report describes a case of VL initially misdiagnosed as brucellosis due to a history of close contact with sheep. The patient tested negative for brucellosis via serum agglutination, blood culture, and bone marrow smear, and showed no improvement with a combination of omadacycline and rifampin therapy. Definitive diagnosis was achieved through metagenomic next-generation sequencing (mNGS) and confirmation with the rk39 antigen test. The patient was successfully treated with amphotericin B cholesterol sulfate complex and recovered fully. This case highlights the need to consider rare pathogens when epidemiological history and clinical response to treatment are incongruent and emphasizes the value of mNGS in timely diagnosis of VL.

Keywords: visceral leishmaniasis, metagenomic next-generation sequencing, brucellosis, fever, splenomegaly

Background

Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania* species. Globally, VL is the second deadliest parasitic disease, with an estimated 50,000 to 90,000 new cases reported annually.[1] According to the Chinese Center for Disease Control and Prevention, VL in China is classified into three types: anthroponotic VL (AVL), mountain-type zoonotic VL (MT-ZVL), and desert-type zoonotic VL (DT-ZVL). Children and farmers are the highest-risk groups for VL.[2]

VL typically manifests after an incubation period with persistent systemic infection, characterized by fever, anemia, and hepatosplenomegaly due to the invasion of *Leishmania* parasites into the reticuloendothelial system, including lymph nodes, spleen, and liver.[3] Diagnosis relies on clinical manifestations, epidemiological history, serological tests, and parasitological examination. Recently, the rk39 antigen rapid test and metagenomic next-generation sequencing (mNGS) have demonstrated high diagnostic value for VL.[4,5]

Here, we report a case of VL initially misdiagnosed as brucellosis based on the patient's epidemiological history and clinical symptoms. The diagnosis was later established using peripheral blood mNGS and confirmed by the rk39 antigen test. The patient was successfully treated with amphotericin B cholesterol sulfate complex and discharged in good condition.

Case Presentation

On August 19, 2023, a 39-year-old male farmer presented with unexplained fever for 15 days, with a maximum temperature of 38.5°C, and no accompanying symptoms. He sought medical attention at a local hospital, where antipyretic treatment temporarily resolved the fever, but it recurred after cessation of medication. On August 29, 2023, the patient developed epigastric pain radiating to the back, accompanied by nausea and vomiting. Abdominal ultrasonography revealed mildly echogenic and punctate hyperechoic findings in the gallbladder, suggestive of possible gallstones or other pathology, along with pancreatic hyperechogenicity and splenomegaly. To determine the etiology, the

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patient was referred to a tertiary hospital in Shijiazhuang on September 3, 2023, where he was admitted to the hepatobiliary surgery department with a preliminary diagnosis of gallstones.

The patient had no significant past medical history and reported good general health. Upon admission, vital signs were as follows: temperature 37.4°C, pulse 102 beats/min, respiratory rate 23 breaths/min, and blood pressure 106/52 mmHg. Physical examination revealed no jaundice, normal cardiopulmonary findings, and a positive Murphy's sign. Laboratory investigations showed elevated liver enzymes: alanine aminotransferase (ALT) 121.3 U/L, aspartate aminotransferase (AST) 49.0 U/L, and gamma-glutamyl transferase (GGT) 116.4 U/L. Chest CT imaging showed no significant abnormalities, while abdominal CT revealed localized thickening of the gallbladder neck wall and splenomegaly (Figure 1). Abdominal ultrasonography confirmed findings of fatty liver, mild hepatomegaly, gallbladder inflammation with possible stones, and splenomegaly (160 mm × 53 mm).

Due to persistent fever with a maximum temperature of 39°C, the patient was transferred to the infectious disease department on September 7, 2023. Epidemiological history revealed contact with over 1000 sheep. The patient did not come into contact with any animals other than sheep. During febrile episodes, the patient reported fatigue, knee, and ankle joint pain. Differential diagnoses included brucellosis and non-infectious fever. He was treated empirically with levofloxacin (0.5 g daily), omadacycline (0.1 g daily), and rifampin (0.6 g daily). Laboratory tests showed a negative Brucella agglutination test, negative vasculitis and antinuclear antibody panels, and negative blood cultures. Complete blood count (CBC) results were as follows: white blood cell count (WBC) 6.18×10^9/L, red blood cell count (RBC) 4.66 $\times 10^{12}$/L, hemoglobin (HGB) 135.00 g/L, and platelet count (PLT) 157.00×10^9/L.

Despite treatment, the fever persisted, peaking at 39.5°C. On September 11, 2023, bone marrow aspiration was performed, Microscopic examination of Giemsa stained bone marrow did not reveal any pathogens. Histopathological evaluation of the biopsy predominantly showed cortical bone with scant bone marrow tissue. Bone marrow cultures were negative.

Given the inconclusive findings from traditional diagnostic methods, peripheral blood was sent to WillingMed Technology (Beijing) Co., Ltd. for metagenomic next-generation sequencing (mNGS). DNA was extracted and prepared for library construction. Sequencing was performed using the MGISEQ-200 platform (MGI Technology) with 50 bp single-end read kits. Quality control of the raw FASTQ data was performed using Trimmomatic v0.40, and high-quality reads aligning to the human genome (GRCh37/hg19) were removed using Bowtie2 v2.4.3. Remaining sequences were annotated using Kraken2 v2.1.0 with the NCBI GenBank database to identify pathogens.[6,7]

On September 13, 2023, mNGS identified *Leishmania infantum* with 8116 unique reads (Figure 2). The Hebei Center for Disease Control and Prevention confirmed the diagnosis on September 20, 2023, through a positive rk39 antigen test. The final diagnosis was visceral leishmaniasis (VL).

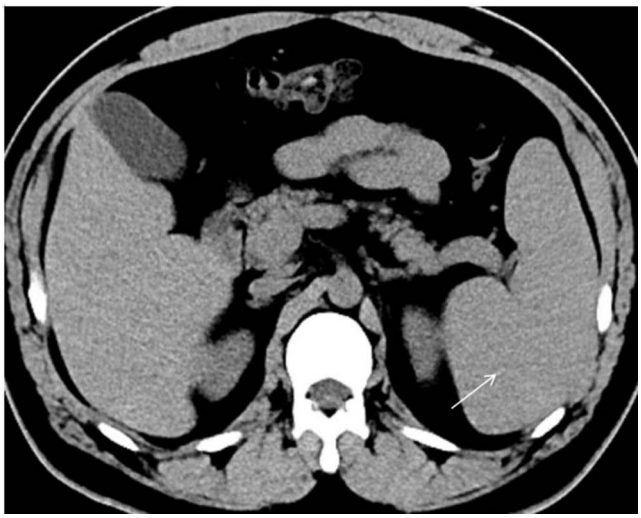

Figure 1 Coronal abdominal computed tomography (CT) scan showing splenomegaly, the white arrow in the picture refers to the spleen.

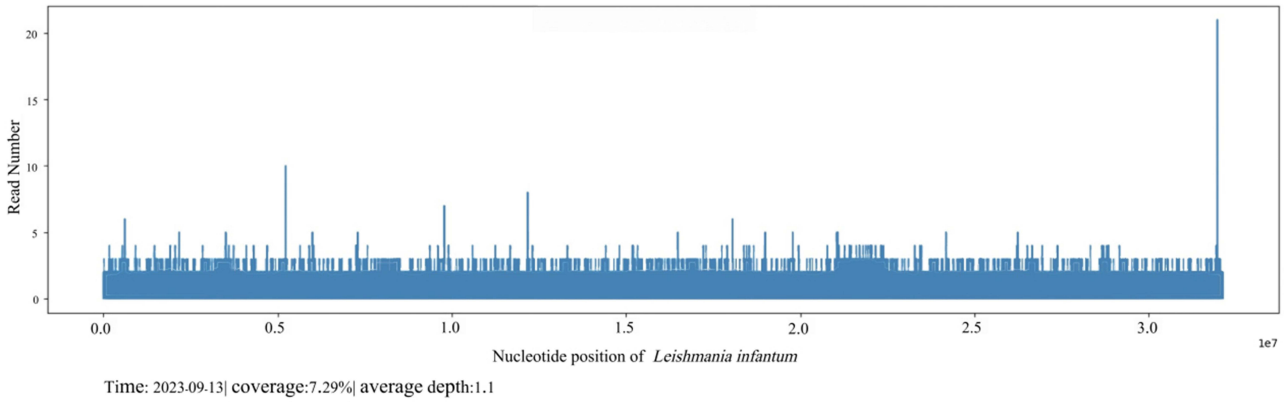

Figure 2 Genome coverage map of *Leishmania infantum* detected by mNGS.

Treatment with amphotericin B cholesterol sulfate complex (ABCD) was initiated on September 13, 2023, with an initial dose of 50 mg on the first day, escalating to 100 mg on the second day, 150 mg on the third day, and 250 mg from the fourth to the seventh day. On the first day of treatment, the patient experienced chills, high fever, and exacerbation of abdominal pain. These symptoms were attributed to gallbladder stone impaction, and pain was managed with phloroglucinol. Percutaneous transhepatic gallbladder drainage was performed under interventional ultrasonography, and cultures of the aspirated fluid were negative. Suspecting an infusion reaction to ABCD, infusion rates were reduced using an infusion pump, which alleviated symptoms.

By September 19, 2023, after a cumulative ABCD dose of 1275 mg, the patient's temperature normalized. Repeat laboratory tests showed improved results: WBC 7.83 × 10^9/L, neutrophil count (NEUT) 4.83 × 10^9/L, RBC 4.25 × 10^{12}/L, HGB 121.00 g/L, PLT 128.00 × 10^9/L, procalcitonin (PCT) 0.314 ng/mL, and C-reactive protein (CRP) 28.00 mg/L.

The patient's condition stabilized, and he was discharged on September 22, 2023. At the 8-month follow-up, the patient remained asymptomatic and in good health.

Discussion

Leishmaniasis is endemic in approximately 100 countries across tropical, subtropical, and temperate regions worldwide.[8,9] In China, the annual incidence of visceral leishmaniasis (VL) is extremely low according to official records. Clinically, VL is characterized by irregular fever, hepatosplenomegaly, cachexia, and pancytopenia.[10] However, its complex and nonspecific clinical presentation often complicates the diagnostic process. *Leishmania* parasites are transmitted via the bites of infected sandflies, subsequently infecting mononuclear phagocytes within the reticuloendothelial system. The patient described in this report had a clear epidemiological history of close contact with sheep, denied travel to endemic areas such as mountains, hills, or deserts, and reported no known insect bites. As the patient sought medical attention in a non-endemic region, the initial suspicion fell on zoonotic brucellosis. The patient's main clinical features included fever with abdominal pain, splenomegaly, and occasional joint pain, without pancytopenia. These symptoms aligned with those of brucellosis. However, a negative Brucella serum agglutination test does not completely exclude the disease. Studies have shown that false-negative results increase with disease duration, exceeding 20% six months post-infection when using tests such as the Rose Bengal Plate Test (RBPT), Gold Immunochromatographic Assay (GICA), and Standard Tube Agglutination Test (SAT).[11] Ultimately, no evidence of brucellosis was found in the pathogen tests, including blood and bone marrow cultures. Additionally, the patient did not respond to treatment with omadacycline and rifampin, which is inconsistent with brucellosis. These findings prompted the use of metagenomic next-generation sequencing (mNGS), which led to a definitive diagnosis of visceral leishmaniasis.

The patient presented with symptoms including fever, splenomegaly, and abdominal pain. However, these clinical manifestations lacked specificity. Similar presentations can be observed in autoimmune diseases such as systemic lupus erythematosus and Sjögren's syndrome, as well as infectious diseases like brucellosis and tuberculosis. Given that the patient resided in a non-endemic region, rare infectious diseases such as visceral leishmaniasis (VL) are easily overlooked, making diagnosis based solely on clinical presentation challenging.[12] It is estimated that for every clinically diagnosed VL case, there are 30–100 subclinical

cases that go unreported due to mild symptoms that do not require hospitalization.[13] Therefore, obtaining direct evidence of *Leishmania* infection is a more accurate and reliable diagnostic approach.[14] Patients with unexplained fever often undergo serological testing for atypical bacterial infections, but negative results do not always exclude the possibility of infection, especially in cases with a clear epidemiological history. When there is inconsistency between standard treatment and clinical syndromes, alternative diagnoses should be considered. This underscores the importance of avoiding rigid clinical thinking. In this case, the patient's symptoms of fever and splenomegaly aligned with VL.[15] Zoonotic VL is commonly caused by *Leishmania infantum*, a pathogen frequently found in dogs and other mammals.[16] A study found that the greatest sandfly density was found in sheep pen.[17] Leishmaniasis can affect many different animals. Dogs are the main hosts of leishmaniasis. The spatial distribution of L. infantum infections in dogs is closely correlated with the presence of the disease in humans;[18] Many wild animals also play the role of hosts. While other indigenous animals, such as marsupials, sloths, and monkeys, are the main hosts for other Leishmania spp.[19] Given that *Leishmania infantum* was identified as the pathogen in this case, it is highly likely that the source of infection was the patient's contact with sheep.

Laboratory testing for visceral leishmaniasis (VL) includes conventional microbiological methods, serological assays, and molecular diagnostics. Detection of *Leishmania* in bone marrow or blood remains the gold standard for VL diagnosis, offering high specificity.[10] While the rK39 IgG antibody test is a routine diagnostic tool for VL, its availability in non-endemic regions is limited.[10] In our case, negative results from blood cultures and bone marrow smears delayed the diagnosis by 10 days. During the early stages of infection, the parasite may not yet have disseminated widely within tissues and organs, and the parasite load may be relatively low. This highlights the limited sensitivity of bone marrow aspiration in diagnosing VL. If *Leishmania* has not invaded the bone marrow, it may be missed, necessitating multiple samples for accurate detection. However, bone marrow aspiration has limitations, including its invasive nature, small sample size, and the need for multiple biopsies, which may be difficult to obtain with patient consent. Additionally, microscopic analysis of bone marrow smears requires significant expertise from clinicians, further limiting its diagnostic utility. As a result, bone marrow biopsy may not always be an effective diagnostic method. Metagenomic next-generation sequencing (mNGS) is a molecular diagnostic tool that enables high-throughput sequencing of clinical samples to identify the nucleic acid sequences of pathogens. This method offers several advantages, including rapid detection, broad pathogen coverage, and high sensitivity and specificity. mNGS has been increasingly applied for the accurate and timely diagnosis of suspected VL cases.[12,20] In our case, the patient's history of contact with sheep initially obscured the suspicion of VL. Negative results from blood cultures and bone marrow smears prompted the use of peripheral blood mNGS, which quickly identified *Leishmania infantum* as the causative pathogen. This provided critical evidence for initiating targeted antiprotozoal therapy, emphasizing the utility of mNGS as a valuable adjunct diagnostic tool for VL.

Based on the diagnostic results, clinicians selected amphotericin B cholesterol sulfate complex (ABCD) for anti-protozoal therapy. However, during the initial treatment phase, the patient experienced chills and high fever. A clinical study involving 30 cases reported an infusion reaction rate of ≥5% with ABCD, including symptoms such as fever, chills, rash, and hypokalemia. By reducing the infusion rate, the patient gradually developed tolerance to the therapy. Therefore, amphotericin B formulations should be administered via slow infusion to minimize infusion-related adverse reactions, enhance treatment efficacy, and reduce patient discomfort.[15]

Conclusion

This case reports Leishmaniasis with clinical manifestations of fever, splenomegaly, and abdominal pain. A history of contact with sheep can easily mask VL, and the patient living in non endemic areas can easily overlook rare infectious diseases, making diagnosis of leishmaniasis difficult. Due to the negative results of blood culture and bone marrow testing, mNGS is an effective auxiliary detection method for leishmaniasis. Sheep may not only be a source of infection for brucellosis, but also for Leishmania parasites in infants. We should be vigilant and take personal protective measures.

Ethics and Consent Statements

This was a retrospective case report study conducted in the Infectious diseases department of a tertiary hospital and was approved by the Hebei General Hospital ethics committee of the hospital (NO. 2025-LW-0037). The patient provided consent for the publication of the case details.

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Disclosure

The authors report no conflicts of interest in this work.

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