



CASE REPORT

Metagenomic next-generation sequencing rescues a case of imported visceral leishmaniasis masquerading as hemophagocytic lymphohistiocytosis

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ABSTRACT

This paper reports a case of imported visceral leishmaniasis (VL; also known as kala-azar) in a non-endemic area (Wuhan). The patient was a 63-year-old male who had recurrent fever for more than 5 months and failed to respond to multiple treatments for hemophagocytic syndrome (HLH). No pathogen was detected by traditional bone marrow aspiration smear microscopy. Finally, 12347 standardized sequence count of *Leishmania* species complex were detected in peripheral blood via metagenomic next-generation sequencing (mNGS), confirming the diagnosis of VL. The patient's condition was relieved after treatment with liposomal amphotericin B. This case suggests that mNGS should be used as a powerful tool for early pathogen screening in patients with fever of unknown origin accompanied by HLH in non-endemic areas.

Keywords: Kala-azar; *Leishmania*; mNGS.

INTRODUCTION

Visceral leishmaniasis (VL) is a zoonotic disease caused by *Leishmania* protozoan infection, with clinical manifestations including prolonged irregular fever, night sweats, weight loss, progressive splenomegaly, pancytopenia and hypergammaglobulinemia (Aggarwal *et al.*, 1999; Safavi *et al.*, 2021). For a long time, the diagnosis of VL has relied on bone marrow aspiration smear microscopy, serological detection and pathogen culture. However, these conventional methods face prominent bottlenecks in clinical practice: the positive rate of bone marrow smears is only 60%–85%, the false negative rate rises significantly in infants, immunocompromised patients or at the early stage of infection, and the invasive and time-consuming operation can hardly meet the demand for rapid diagnosis (Daneshbod *et al.*, 2010; Vijayakumar *et al.*, 2024). Although serological tests are easy to perform, they may have cross-reactions with antigens of other pathogens, leading to a high false positive rate (Sakkas *et al.*, 2016), and false negative cases are also reported simultaneously (El-Amin *et al.*, 2020). Especially in non-endemic areas or cases with atypical clinical manifestations, routine laboratory tests often confuse VL with hematological diseases, thus delaying the critical treatment (Zhang *et al.*, 2026; Zhu *et al.*, 2026). In recent years, mNGS, with its unbiased, high-sensitivity and broad-spectrum pathogen detection capability, has paved a new path for the accurate diagnosis of VL (Chen *et al.*, 2020; Song *et al.*, 2021). This article reports the diagnosis and treatment experience of an imported VL patient in a non-endemic area who was identified via mNGS.

CASE PRESENTATION

A 63-year-old male, residing in Wuhan, Hubei Province, China, with native place in Yangquan, Shanxi Province, China, presented

with recurrent fever for more than 5 months. On January 2, 2026, the patient developed fatigue, chills and poor appetite without obvious inducement, and sought medical attention at Hospital A in Wuhan. Laboratory tests showed WBC $4.52 \times 10^9/L$, Hb 77 g/L, PLT $62 \times 10^9/L$; erythrocyte sedimentation rate 35 mm/h; D-dimer 59.53 µg/ml; procalcitonin 2.27 ng/ml, high-sensitivity C-reactive protein 132.47 mg/L; interleukin-6 52.33 µg/ml; aspartate aminotransferase 82 U/L, creatinine 244 µmol/L, triglyceride 2.59 mmol/L. The initial diagnosis considered bacterial infection, renal insufficiency, elevated transaminase and abnormal coagulation function.

The patient was transferred to Hospital B in Wuhan on January 10, 2026. PET-CT examination revealed multiple splenic infarcts, splenomegaly, and diffuse increased glucose metabolism in the spleen; diffuse mild elevation of bone marrow glucose metabolism within the detection range, which was considered to be caused by hemophagocytic syndrome. No obvious signs of malignant tumors were found in other detected sites. Bone marrow aspiration showed active bone marrow hyperplasia, with phagocytes accounting for 3.5% and hemophagocytosis observed. The patient presented with fever, splenomegaly, pancytopenia, hemophagocytes in bone marrow, elevated sCD25 and ferritin, and hemophagocytic syndrome was considered for diagnosis. No significant abnormality was detected in the gene mutation test for hemophagocytic syndrome. During hospitalization, the patient received 2 sessions of plasma exchange, methylprednisolone and ruxolitinib, with slightly improved body temperature. The patient continued oral methylprednisolone and ruxolitinib outside the hospital to control the condition.

The patient received multiple consecutive consultations at Hospital B from March 5, 2026 to May. Anti-infective therapy with cefoperazone-sulbactam, glucocorticoids, and ruxolitinib for disease control were administered sequentially, yet no clinical improvement

was observed. On May 10, 2026, the patient developed recurrent fever after glucocorticoid withdrawal, with a maximum body temperature of 39°C. The patient was admitted to our hospital via the outpatient department with a diagnosis of “hemophagocytic lymphohistiocytosis (HLH)” for further diagnosis and treatment. All previous laboratory results of the patient are shown in Table 1. Given that the patient had received multiple consultations in external hospitals, with relevant laboratory indicators meeting the diagnostic criteria for HLH, the therapeutic effect remained poor for multiple times and the primary etiology was never clarified. Considering this patient as a difficult case of HLH of unknown origin, after the departmental difficult case discussion, it was decided to collect the patient’s blood sample for mNGS of pathogenic microorganisms to identify the infectious etiology.

Introduction to the mNGS workflow

DNA Extraction and Library Construction

Peripheral blood specimens were collected from the patient, followed by centrifugation at 4,000 rpm for 10 minutes. 600 µL of the supernatant was then collected, and total DNA was extracted using a DNA extraction kit (2005, Genskey, Tianjin) with an elution volume of 50 µL. The extracted DNA was sonicated to generate fragments of 200–300 bp. An NGS library preparation kit (2102, Genskey, Tianjin) was used for end repair, and unique molecular identifiers (UMIs) were introduced via adapter ligation, which was applied for duplicate correction in downstream PCR amplification and subsequent PCR amplification procedures. After concentration quantification, the libraries were pooled and subjected to single-end 50 bp (SE50) sequencing on the MGISEQ-200 sequencing platform, with the raw data output per sample no less than 20 M reads.

Bioinformatic Analysis

Raw sequencing data were quality-controlled and filtered using fastp (v0.22.0). Reads meeting any of the following criteria were excluded: (1) reads with the proportion of low-quality bases (quality value < 15) ≥ 40%; (2) reads containing more than 5 N bases; (3) reads mapped to

sequencing primer sequences. Meanwhile, PCR duplicate sequences were removed to obtain high-quality usable data. Subsequently, high-quality reads were aligned to the host genome database (integrated T2T genome and NCBI human reference sequences) via bowtie2 (v2.3.5.1) to filter out reads mapped to human sequences, and the remaining non-human high-quality sequences were used for downstream microbial identification. The non-human sequences were aligned to a self-constructed microbial genome database for species classification using bwa (v0.7.17-r1198-dirty).

Microbial Database and Positive Interpretation Criteria

The species classification database is integrated from NCBI RefSeq, NCBI GenBank, FDA-ARGOS, Genome Taxonomy Database (GTDB) and the Influenza Virus Database, covering 12,895 species of bacteria, 11,120 species of viruses, 1,582 species of fungi, 312 species of parasites, 177 Mycobacterium complexes and 184 Mycoplasma/Chlamydia species.

The principles for database construction are as follows: representative genomes are selected with priority; if no representative genome is available, the genome with the longest contig N50 is selected; if the intra-species genome similarity is less than 95%, multiple genomes are included according to the genome quality and similarity.

The positive judgment criteria are as follows: the number of species-specific reads, after being normalized to 1 million usable reads, is ≥ 10, and the number of specific primer matches is ≥ 1; if the same pathogen is detected in the negative control, the read count of the sample needs to be ≥ 10 times that of the negative control to be judged as positive.

mNGS Detection Results

On May 15, 2026, pathogen mNGS detected *Leishmania donovani* species complex, with a standardized sequence number of 72,776 and a relative abundance of 99.99%; the species-level identification was *Leishmania infantum*, with a standardized sequence number of 1,115 and a relative abundance of 92.49%. The distribution of the detected gene sequences is shown in Figure 1.

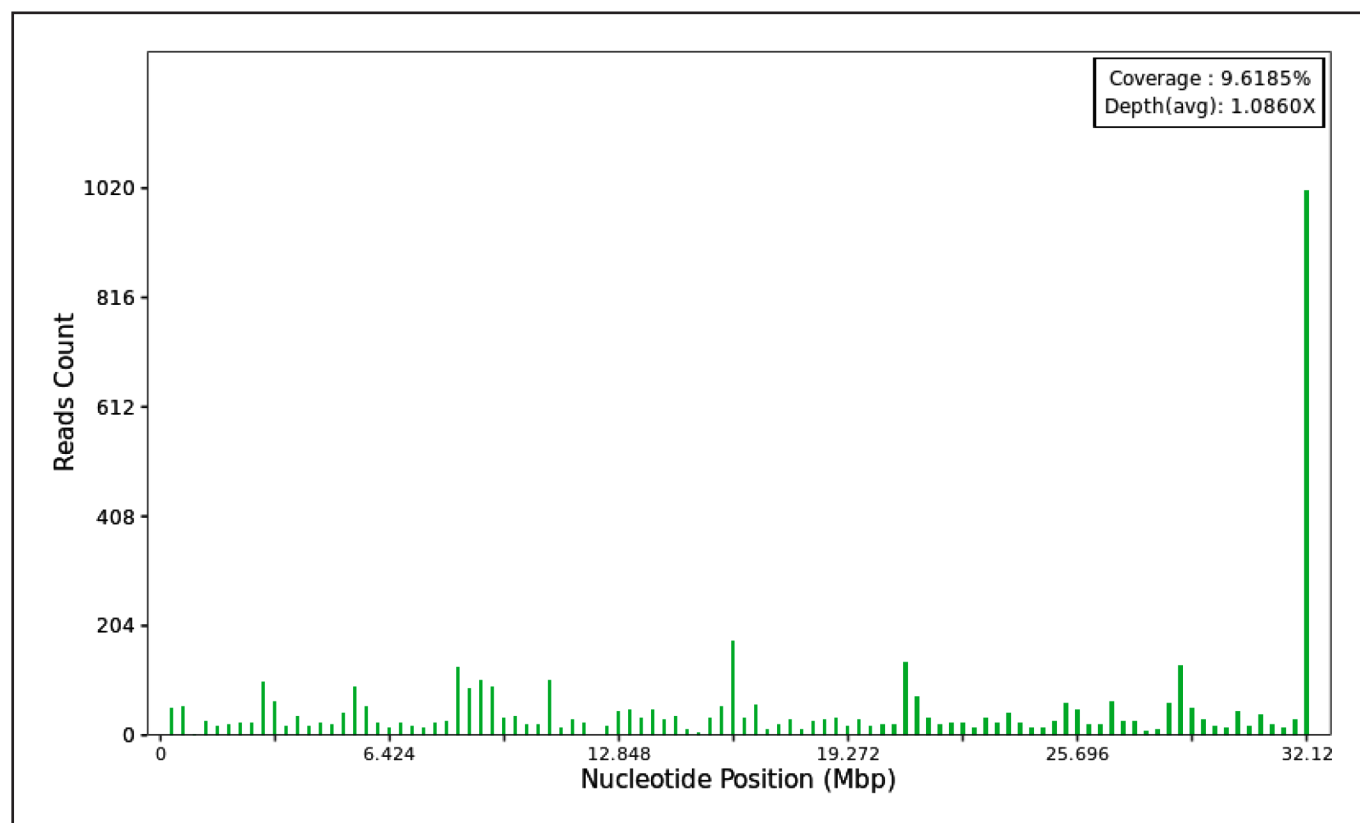


Figure 1. Distribution map of detected gene sequences of *Leishmania infantum*.

Bone Marrow Cytological Examination Findings

On May 16, 2026, bone marrow cytology revealed *Leishmania* parasites and hemophagocytes, with non-Hodgkin lymphoma (NHL) to be excluded (Figure 2). Bone marrow biopsy showed uneven bone marrow hyperplasia, all three lineages of hematopoietic cells were observable, and granular inclusions were detected in partial cytoplasm. Combined with bone marrow smear and clinical laboratory tests, the findings were consistent with *Leishmania* infection.

Treatment and Prognosis

The patient had recurrent episodes of HLH and immunodeficiency. This time, the patient presented with extremely elevated CRP and PCT, as well as pulmonary infection, so enhanced anti-infective therapy was administered. The confirmed *Leishmania* infection was

considered a potential trigger for HLH, and liposomal amphotericin B was prescribed in accordance with guideline recommendations. Since there is insufficient evidence for the patient's current tumor, and the patient has multiple infections, HLH-targeted chemotherapy was temporarily withheld, and intravenous immunoglobulin combined with glucocorticoids was administered to ameliorate HLH. Electrolyte disturbance was corrected, renal function was improved, and complete blood count, biochemistry and DIC markers were closely monitored for timely symptomatic management. On May 20, 2026, the patient's general condition improved and was discharged as advised, with continued liposomal amphotericin B therapy outside the hospital. Subsequent outpatient reexamination showed that the laboratory indicators gradually returned to normal, as shown in Table 1.

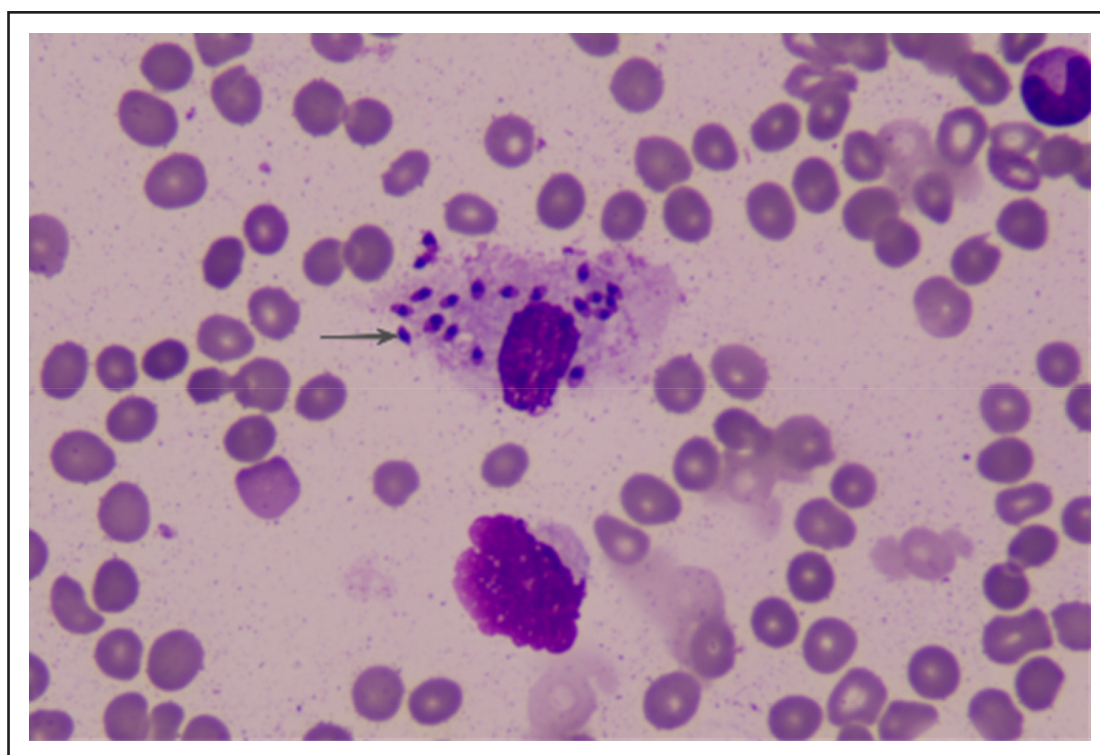


Figure 2. Amastigote of *Leishmania* (bone marrow, the arrow indicate the characteristic kinetoplast).

Table 1. Changes in laboratory indicators

Date	WBC ($\times 10^9/L$)	Hb (g/L)	PLT ($\times 10^9/L$)	Ferritin (ng/ml)	sCD25 (pg/ml)	Creatinine ($\mu\text{mol/L}$)
2026/1/2	4.52	77	62	/	/	244
2026/1/10	1.15	78	51	24704.09	56186	/
2026/1/28	/	/	/	1442.15	/	/
2026/3/5	5.19	105	149	3502.84	/	/
2026/3/11	/	/	/	1479	/	/
2026/4/2	/	/	/	4071.31	17112	/
2026/4/16	1.4	87	75	7885.91	/	/
2026/4/22	0.84	79	30	3086.83	/	/
2026/5/10	3.24	80	48	40000	2602	432.7
2026/7/14	4.41	114	171	580.9	495.7	157.5
2026/8/18	5.39	120	177	189.6	/	131.1

Note: “/” indicates that the relevant examination was not performed on that day.

DISCUSSION

Visceral leishmaniasis also known as VL, is a zoonotic parasitic disease. In China, the main pathogens causing VL are *L. donovani* and *L. infantum* (Huang et al., 2025). Its transmission vector is the female sandfly, and its life cycle consists of two morphological forms: amastigote and promastigote. The amastigote, also termed Leishman-Donovan body, parasitizes in the mononuclear macrophages of humans and mammals; the promastigote resides in the digestive tract of sandflies. Currently, VL maintains an overall low-endemic trend in China, yet it has shown an increasing incidence in recent years. In 2024, the main endemic counties (districts, cities) of VL in China included Pingding County in Shanxi Province, Linzhou City in Henan Province, Jingxing County in Hebei Province, the Suburban District of Yangquan City, and Dengfeng City in Henan Province.

The main clinical manifestations of VL include prolonged irregular fever, night sweats, weight loss, progressive splenomegaly, mild to moderate hepatomegaly, pancytopenia and hypergammaglobulinemia, as well as occasional symptoms such as epistaxis and gingival bleeding. Due to insufficient awareness of this disease among clinicians in non-endemic areas, it is easily misdiagnosed as “hematological diseases”, “autoimmune diseases” and other diseases, leading to poor therapeutic effects and protracted disease course.

The diagnostic principle of VL should be formulated based on the epidemic history, clinical manifestations and laboratory test results (Aggarwal et al., 1999). Traditional laboratory detection methods include etiological diagnosis and serological testing. Etiological diagnosis serves as the gold standard, but the detection sensitivity of bone marrow aspiration smear is only about 60%–85% (Daneshbod et al., 2010), with a risk of missed diagnosis. Protozoan culture has high sensitivity but requires a long incubation period. Serological tests include direct agglutination test, immunochromatographic assay and ELISA. Although these methods are easy to operate, they may have cross-reactions with antigens of other pathogens, resulting in a relatively high false positive rate (Aronson & Satoskar, 2026).

In this case, Wuhan is a non-endemic area of VL, while the patient’s native place is Yangquan, Shanxi Province, so this case is defined as an imported infection. The patient first sought medical attention with fever, fatigue and poor appetite. Laboratory tests showed elevated ferritin, splenomegaly, and two-lineage cytopenia (hemoglobin and platelet count). Bone marrow smear examination revealed hemophagocytosis, and the patient met the diagnostic criteria for hemophagocytic syndrome according to the HLH-2004 guideline (Henter et al., 2007). However, neither glucocorticoid therapy nor targeted drug treatment based on the HLH treatment guideline achieved satisfactory efficacy, and the underlying etiology was not identified. Although the patient underwent multiple bone marrow aspiration examinations, no *L. donovani* parasites were detected under the microscope, due to the uneven distribution of Leishmania parasites in bone marrow, low parasite load at the initial onset, and high technical requirements for laboratory personnel.

In recent years, with the continuous development of molecular biological detection technologies, mNGS can unbiasedly detect a wide range of pathogenic microorganisms through sequencing and analysis of microbial and host nucleic acids in clinical samples. Owing to its advantages of unbiased detection, high throughput and no need for pre-set pathogen assumptions, it has significantly improved the etiological diagnostic capability for clinically difficult and complicated infections (Chen et al., 2020; Song et al., 2021). In contrast, the targeted PCR detection technology has lower cost, faster detection speed, and comparable sensitivity and specificity compared with mNGS. However, this technology requires clinicians to have a suspected diagnosis before sample submission, so that specific pathogen targets can be pre-selected. In non-endemic areas of the disease, clinical laboratories are not equipped with targeted PCR reagents, making it difficult to achieve immediate

sample submission in practical operations. In comparison, mNGS takes the core concept of hypothesis-free and full-coverage, which can complete broad-spectrum screening in a single experiment without pre-setting pathogens. In this case, after admission to our hospital, the patient underwent pathogen mNGS testing due to long-term “fever of unknown origin”, and the etiology was identified within 24 hours, which enabled the patient to receive effective treatment. Sequencing analysis of the patient’s peripheral blood plasma identified reads belonging to the *L. donovani* species complex, among which 1115 reads were definitively assigned to *L. infantum*. This markedly elevated number of detected sequences indicates a high parasitic load in the patient’s peripheral blood. This phenomenon may be closely correlated with the patient’s exceptionally severe HLH phenotype, protracted disease course, and poor response to conventional immunosuppressive therapy, which may also account for the development of the patient’s severe and persistent HLH phenotype. Notably, the mNGS result of this patient clearly identified *L. infantum*, the main pathogen of canine-type VL in mountainous areas of northern China. This finding is highly consistent with the epidemiological background of the patient’s native place Yangquan City, Shanxi Province (one of the current hilly endemic foci), which further confirms the source of this imported infection case. Traditional bone marrow smear microscopy cannot distinguish different Leishmania species, while mNGS, with its high-resolution pathogen identification capability, not only assisted the clinical diagnosis in this case, but also provided critical evidence for epidemiological traceability. Furthermore, the quantitative potential of mNGS (e.g., changes in normalized read counts) also provides novel insights for therapeutic response monitoring. Previous studies have demonstrated that the nucleic acid load of pathogens in peripheral blood declines following effective anti-infective therapy. In this case, the patient continued to receive liposomal amphotericin B after discharge, and both clinical symptoms and laboratory parameters (complete blood count, ferritin, sCD25, renal function) showed a sustained improving trend during follow-up (Table 1). Although repeated mNGS testing was not performed during treatment, dynamically monitoring the changes in pathogen sequence counts via peripheral blood mNGS in future clinical practice may serve as a non-invasive auxiliary method to evaluate anti-*Leishmania* therapeutic efficacy and determine pathogen clearance, which presents unique advantages especially for patients who cannot tolerate repeated bone marrow aspiration. Naturally, the correlation between mNGS quantification and clinical outcomes still requires validation in larger-sample studies.

In conclusion, in non-endemic areas, due to insufficient awareness and reduced vigilance of clinicians against VL, coupled with the limited sensitivity of traditional etiological detection methods that heavily rely on operational experience, the disease is often misdiagnosed as hematological disorders or autoimmune diseases with a high misdiagnosis rate, leading to delayed treatment. Metagenomic sequencing, with its unique technical advantages, enables early and rapid identification of infectious pathogenic microorganisms, and presents higher diagnostic value especially for pathogens that grow slowly or are difficult to culture, such as parasites. This case fully demonstrates that for patients with HLH of unknown etiology, mNGS testing should be adopted as early as possible to rule out infectious etiologies — especially for patients who do not respond to immunosuppressive therapy, since delayed anti-parasitic treatment may lead to severe consequences. In addition, for difficult infectious cases with fever of unknown origin, ineffective conventional anti-infective and immunosuppressive therapy, and repeatedly negative results of traditional microbiological examinations, mNGS testing can also be used as an important supplementary method or first-line screening tool, which significantly shortens the diagnostic cycle and guides precise treatment. With intensified globalization and population mobility, imported epidemic infection cases in non-endemic areas

will keep increasing. The popularization and application of mNGS technology is expected to compensate for the cognitive gap of clinicians on rare pathogenic microorganisms, and provide strong technical support for breaking through the diagnostic blind zone of fever of unknown origin.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Not applicable.

REFERENCES

- Aggarwal, P., Handa, R., Singh, S. & Wali, J.P. (1999). Kala-azar – new developments in diagnosis and treatment. *Indian Journal of Pediatrics* **66**: 63-71.
- Aronson, N.E. & Satoskar, A.R. (2026). Leishmaniasis. *New England Journal of Medicine* **394**: 2026-2039. <https://doi.org/10.1056/NEJMra2501234>
- Chen, H., Fan, C., Gao, H., Yin, Y., Wang, X., Zhang, Y. & Wang, H. (2020). Leishmaniasis diagnosis via metagenomic next-generation sequencing. *Frontiers in Cellular and Infection Microbiology* **10**: 528884. <https://doi.org/10.3389/fcimb.2020.528884>
- Daneshbod, Y., Dehghani, S.J. & Daneshbod, K. (2010). Bone marrow aspiration findings in kala-azar. *Acta Cytologica* **54**: 12-24. <https://doi.org/10.1159/000324968>
- El-Amin, L.M., Khalid, K.E. & El-Badry, A.A. (2020). Risk-free point-of-care visceral leishmaniasis diagnostics: combining buffy coat microscopy and immunoassay in tertiary rural hospitals in Sudan. *Acta Tropica* **211**: 105599. <https://doi.org/10.1016/j.actatropica.2020.105599>
- Henter, J.I., Horne, A., Aricó, M., Egeler, R.M., Filipovich, A.H., Imashuku, S., Ladisch, S., McClain, K., Webb, D., Winiarski, J. et al. (2007). HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatric Blood & Cancer* **48**: 124-131. <https://doi.org/10.1002/pbc.21039>
- Huang, L.L., Liu, J.S., Zhou, Z.B., Zhang, Y., Wang, L., Chen, X., Li, H., Yang, F., Wu, J. & Zhao, Q. (2025). Epidemiological characteristics analysis of canine-origin visceral leishmaniasis in China from 2011 to 2023. *Journal of Tropical Diseases and Parasitology* **23**: 131-136.
- Jia, Y.Q., Feng, X.F., Bai, X.B., Li, M., Wang, S., Zhang, L., Chen, Y., Liu, H., Yang, J., Wu, T. et al. (2025). Research progress on kala-azar. *Chinese Journal of Infectious Diseases* **43**: 563-569.
- Safavi, M., Eshaghi, H. & Hajihassani, Z. (2021). Visceral leishmaniasis: Kala-azar. *Diagnostic Cytopathology* **49**: 446-448. <https://doi.org/10.1002/dc.24689>
- Sakkas, H., Gartzonika, C. & Levidiotou, S. (2016). Laboratory diagnosis of human visceral leishmaniasis. *Journal of Vector Borne Diseases* **53**: 8-16.
- Song, P., Chen, S., Tan, X., Gao, Y., Fu, J., You, Z., Wang, C., Zhao, Q. & Pang, F. (2021). Metagenomic analysis identifying a rare Leishmania infection in an adult with AIDS. *Frontiers in Cellular and Infection Microbiology* **11**: 764142. <https://doi.org/10.3389/fcimb.2021.764142>
- Vijayakumar, S., Narayan, P.K., Kumari, S., Ranjan, R., Kumar, V., Kumar, A. & Alti, D. (2024). A review of non-invasive samples and tools in kala-azar diagnosis and test of cure. *Experimental Parasitology* **259**: 108713. <https://doi.org/10.1016/j.exppara.2024.108713>
- Zhang, C.L., Xu, B., Zhu, D.Y., Li, J., Wang, H., Chen, Z. & Liu, X. (2026). One case of kala-azar misdiagnosed as multiple myeloma. *Chinese Journal of Clinical Laboratory Science* **44**: 396-398.
- Zhu, X., Wu, X., Zhou, M.Y., Wang, L., Chen, J., Yang, S. & Zhao, H. (2026). Case analysis of imported visceral leishmaniasis with misdiagnosis and mistreatment leading to multiple organ failure complicated by hemophagocytic syndrome. *Clinical Misdiagnosis & Mistherapy* **39**: 1-7.