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Diagnostic Value of Cerebrospinal Fluid Metagenomics Next-generation Sequencing in Neurobrucellosis in Children

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Objective: To explore the clinical characteristics of neurobrucellosis in children and the diagnostic value of metagenomics next-generation sequencing (mNGS) of cerebrospinal fluid and traditional microbial detection methods.
Methods: Three patients in the pediatrics department from April 2022 to October 2023 were diagnosed with brucellosis, and 5 mL of cerebrospinal fluid was taken and sent for routine biochemical, bacterial Gram-stained smear, antacid-stained smear, ink staining, bacterial culture and second-generation sequencing of the microgeneration of the cerebrospinal fluid, respectively (Beijing AJiAn Genetics Medical Laboratory). Cranial magnetic resonance imaging, blood culture and blood Brucella antibody test were also performed to summarize the clinical features and pathogenic analysis.
Results: The chief presentations were fever, headache, vomiting, somnolence and positive signs of meningeal irritation in all patients. Case 3: persistent hemiparesis of the left limb and cerebral infarction. Brucella was detected by cerebrospinal fluid mNGS in all cases, with sequence numbers of 5252, 162 and 59 Brucella and relative abundances of 80.26%, 6.14% and 1.06%, respectively. Cerebrospinal fluid cultures were negative, and blood cultures were positive in case 3.
Conclusions: The clinical characteristics of neurobrucellosis in children are variable, and meningoencephalitis is common. Traditional microbiological tests are difficult to detect Brucella, whereas cerebrospinal fluid mNGS can provide a precise diagnosis.

Key Words: metagenomics, next-generation sequencing, neurobrucellosis, children

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Brucellosis is a common zoonotic infectious disease worldwide and is referred to as neurobrucellosis when it affects the nervous system. The incidence of pediatric neurobrucellosis is reported to be only 0.8%, with clinical manifestations that are highly variable and prone to misdiagnosis. Some cases may result in severe sequelae, including deafness, paralysis or death.[1,2] Diagnosis relies on bacteriologic or serologic testing, but the sensitivity of routine culture methods is limited. Recently, cerebrospinal fluid (CSF) metagenomics next-generation sequencing (mNGS) has been introduced for diagnosing neurobrucellosis,[3] demonstrating greater sensitivity and specificity compared with conventional diagnostic approaches. This study analyzed the clinical features and CSF mNGS results of 3 neurobrucellosis cases to objectively assess the utility of mNGS for precise diagnosis.

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MATERIALS AND METHODS

Patients and Ethical Clearance

Three neurobrucellosis patients were retrospectively analyzed from May 2022 to October 2023. Detailed records of medical history, physical signs and examination findings were collected. The study was approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University.

Inclusion Criteria[4]

(1) Signs and symptoms of neurological infection, CSF examination showing lymphocytosis, increased protein and normal or decreased glucose; (2) identification of Brucella: antibody titer ≥1:160 from serum and/or CSF using a standardized agglutination test and/or CSF positive for Brucella antibodies and (3) epidemiologic history: exploration of domestic animals or livestock products before the onset of illness or residents living in the infected area.

Exclusion Criteria

(1) Clinical evidence of serologic or culture or isolation of other microorganisms, such as bacteria, viruses, fungi or parasites, causing central nervous system (CNS) infections and (2) Noninfectious encephalitis, such as autoimmune encephalitis.

Examination

Performed blood Brucella antibody (Standard Tube Agglutination Test and Rose-Bengal Plate Agglutination Test), Brucella antibody titer (tube agglutination method), CSF routine, biochemistry, ink staining, acid-fast staining, cytokine test, CSF and blood culture and cranial magnetic resonance imaging (MRI).

Cerebrospinal fluid mNGS detection

Three milliliters of CSF were collected after lumbar puncture, and 300 μL of CSF was taken from the patient and mixed with glass beads and lysate to break the wall. After 10 min, pathogenic microorganisms were detected using the magnetic bead method. DNA/RNA Extraction Kit (NG550-TD, Tian gen Biochemical Technology Co., Ltd., Beijing, China) was used for DNA extraction. The extracted DNA was used for DNA library construction. DNA libraries were constructed according to the kit instructions. The Agilent 4150 Tape Station was used for quality control of the library, with a fragment size of 350–400 bp, and the Qubit DNA HS Assay Kit (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) was used to determine the DNA library concentration. Samples that passed quality inspection were subjected to library denaturation after proportional mixing, and high-throughput sequencing was performed using MGI 200.

Data Analysis

After data generation and analysis, the raw data were extracted, split and processed to filter out low-quality reads and

remove adapters. The filtered data were aligned to the human genome using Burrows-Wheeler Alignment to exclude human-derived sequences and repetitive elements. The remaining data were analyzed using Kraken2 and BLAST, comparing them with an integrated microbial database that included bacteria (17,157 species), fungi (903 species), viruses (8,472 species), parasites (288 species) and spirochetes (89 species). The results provided the number of detected microorganisms and their sequences, which, combined with clinical information, were used to identify potential pathogens.

RESULTS

Case 1

Case 1 involved a female patient 3 years and 6 months old who presented with dizziness, vomiting and drowsiness and was diagnosed with neurobrucellosis. On physical examination, neck stiffness was noted, while muscle strength and tone were normal. Results of the CSF examination are shown in Table 1. On May 7, 2022, a Brucella antibody test (plate agglutination and Rose-Bengal plate agglutination methods) was repeated, showing an antibody titer of 1:200 (+++) using the tube agglutination method. Cranial MRI with FLAIR revealed the following findings: (1) Abnormal enhancement on the right side of the optic chiasm. (2) Suspected subacute infarction of the right cerebral peduncle. (3) A few demyelinating lesions in the brain (Fig. 1). The mNGS results are presented in Table 2. The patient had a history of residence in Inner Mongolia and prior contact with sheep. Treatment included meropenem for 1 month followed by compound sulfamethoxazole and rifampicin for 4 months. However, the patient experienced persistent left hemiplegia.

Case 2

Case 2 involved a 14-year-old female patient admitted with headache, vomiting, lethargy and weakness in the lower limbs. She was diagnosed with a headache and CNS infection. Physical examination revealed neck stiffness. The muscle strength of the right limb was grade 5, and the left limb was grade 5−. Muscle tone was normal in all extremities. CSF examination results are shown in Table 1. On September 15, 2022, a repeat Brucella antibody test using the same methods as in Case 1 returned positive results. Cranial MRI with FLAIR showed no abnormalities. The mNGS results are presented in Table 2. The patient also had a history of residence in Inner Mongolia and prior contact with sheep. Treatment included intravenous ceftriaxone combined with doxycycline and rifampicin for 4 months. After continuing regular treatment with ceftriaxone, compound sulfamethoxazole, rifampicin and doxycycline for 2 months, the patient recovered fully without any sequelae.

Case 3

Case 3 was a 14-year-old male admitted to the hospital with headache, vomiting and lethargy. Physical examination revealed a soft neck, normal muscle strength and tone and no pathological reflexes. The results of the CSF examination are shown in Table 1. On September 13, 2022, a repeat Brucella antibody test using the same method as in Case 1 returned positive results. Cranial MRI with Fluid-attenuated Inversion recovery showed no abnormalities. The results of mNGS are presented in Table 2. The patient had a history of residence in Inner Mongolia and prior contact with sheep too. Treatment included intravenous administration of ceftriaxone, doxycycline, rifampicin and compound sulfamethoxazole for 4 months, followed by oral administration of compound sulfamethoxazole, rifampicin and doxycycline for 2 months. The patient improved without any sequelae.

DISCUSSION

The clinical manifestations of neurobrucellosis are varied and may include meningoencephalitis, myelitis, cerebrovascular disease, peripheral neuropathy and psychiatric disorders.[5] The underlying pathophysiological mechanisms involve direct hematogenous dissemination of the bacteria and immune-mediated processes. Astrocyte proliferation and reactive microglia activation trigger an inflammatory response, producing large amounts of proinflammatory mediators, including reactive oxygen species, NO (nitric oxide), Tumor Necrosis Factor-α (TNF-α) and Interleukin-1(IL-1), which ultimately result in neuronal death.[6]

The most common presentation of neurobrucellosis in children is meningitis, with or without encephalitis. In this study, the patients exhibited meningitis with encephalitis and cerebral infarction, indicating that neurobrucellosis can cause damage to the meninges, brain parenchyma and cerebrovascular structures, consistent with previous reports.[7] CSF inflammatory markers, IL-6, IL-10 and TNF-α, were significantly elevated, suggesting an inflammatory response within the CSF. The levels of these markers decreased gradually with treatment, which has been less frequently reported in the literature.[8–10] Neuroimaging findings in neurobrucellosis are highly variable.[11,12] In case 1, cranial MRI revealed an inflammatory pseudotumor near the right side of the optic chiasm and acute infarction in the right basal ganglia and thalamus, findings consistent with the literature. Although MRI findings improved significantly after treatment, the patient experienced impaired limb movement, likely due to delayed initiation of therapy. These findings underscore the importance of timely diagnosis and appropriate treatment.

Laboratory tests for CNS neurobrucellosis include bacterial culture (blood and CSF), serum immunology, PCR and other methods.[13] A positive blood or CSF culture for Brucella remains the "gold standard" for diagnosis, but the process is time-consuming, with low specificity (20–28%) and sensitivity (37%).[14] Reported positivity rates for blood and CSF cultures are 28% and <20%, respectively.[15,16] In this study, CSF cultures were negative, while blood cultures were positive in case 3, aligning with findings in previous reports. The low sensitivity of culture-based methods is likely due to limitations in laboratory techniques and the low bacterial load in CSF.[17] Serum immunological tests for Brucella

TABLE 1. Results of CSF Examinations

	Date of CSF Collection	White blood cell Count	Monocyte Count	Polymorphonuclear Count	Glucose	Protein	Chloride	IL-10	IL-6	Bacterial Culture of CSF
Reference range		0–5 (/mm^3)			2.5–4.5 (mmol/L)	0.15–0.45 (g/L)	120–130 (mmol/L)	0–4.9 (pg/dL)	0–16.6 (pg/dL)	(–)
Case 1	2022.05.05	106.00	41.00	59.00	1.00	1.093	120	121.14	4851.70	(–)
Case 2	2022.09.15	525.00	99.00	1.00	2.85	1.445	117.0	6.15	330.99	(–)
Case 3	2022.09.01	408.00	97.90	2.10	2.03	0.769	113.0	23.37	619.78	(+)

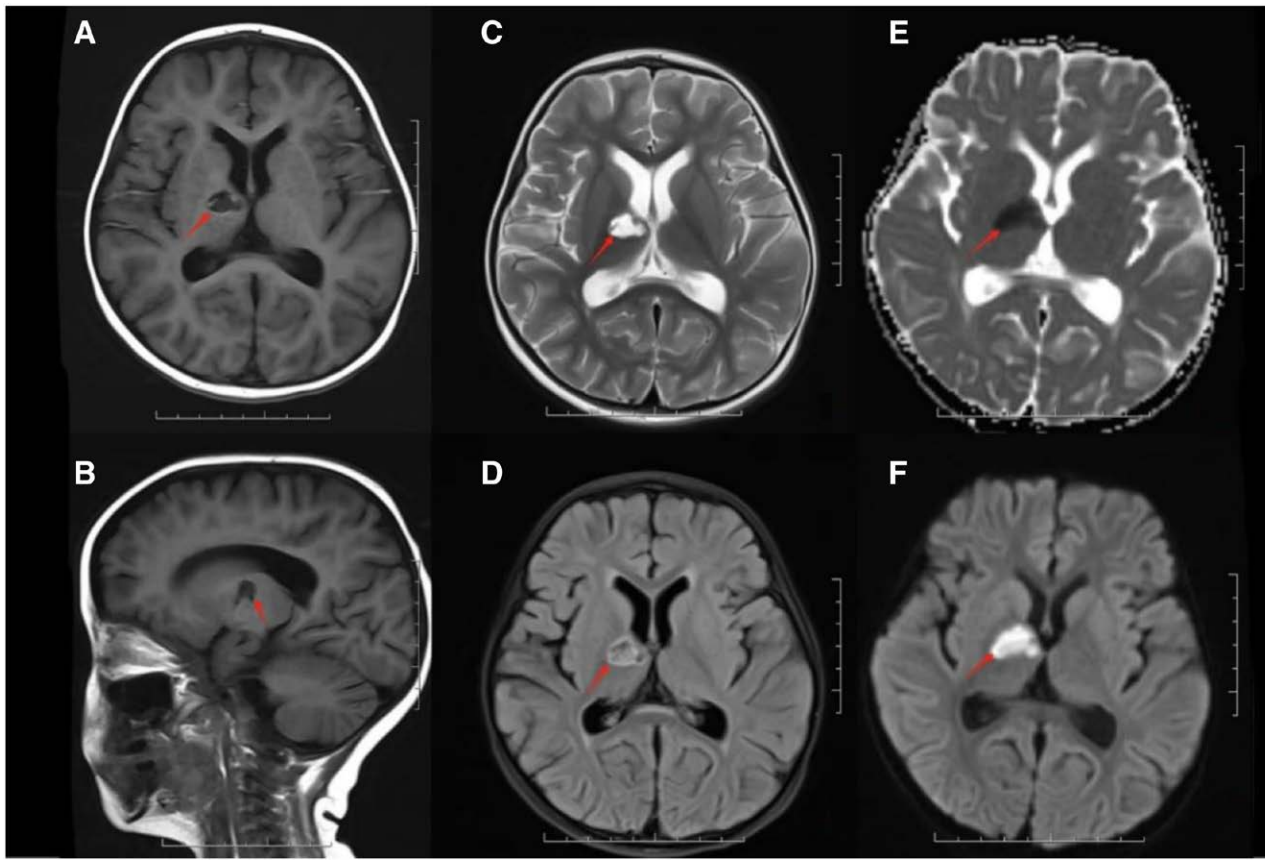

FIGURE 1. Abnormal cranial MRI findings in Case 1. A–C: Irregularly shaped T1-hyperintensity signals are observed in the right basal ganglia and thalamus. D: FLAIR showed hyperintensity signal. E,F: DWI showed hyperintensity signal, ADC showed hypointensity signal.

TABLE 2. Results of mNGS of CSF in Case 1

Case Number	Q30 Proportion (%)	The Detected Sequence of Brucella SPP	Relative Abundance (%)	Coverage (%)	Sequencing Depth (X)	Clinical Diagnosis
1	92.36	162	6.14	1.265	1.00	Brucella melitensis meningitis
2	94.48	5252	80.26	4.340	1.00	Brucella melitensis meningitis
3	93.95	59	1.06	0.045	1.00	Brucella melitensis meningitis

antibodies were positive in all patients, with antibody titers of 1:200, providing critical diagnostic evidence. These tests are relatively simple and rapid but cannot distinguish between active and past infections and may occasionally yield false-negative results.[17] Polymerase Chain Reaction is a valuable diagnostic tool for neurobrucellosis, but it is time-consuming and less practical in emergency settings.

mNGS is a rapidly advancing technology characterized by high throughput, speed and accuracy[18]. It does not require primers or probes, making it particularly suitable for detecting rare, uncommon or complex pathogens. mNGS has become a key method for identifying the causative agents of CNS infections. It can detect all pathogens with DNA or RNA as their genetic material, including Brucella, which has DNA. The sensitivity of CSF mNGS in diagnosing encephalitis and meningitis is reported to be 73%, with a specificity of 99%.[19] In this study, mNGS results were positive for all 3 patients, significantly aiding in diagnosis and guiding treatment. The sequencing data showed abnormal results, and the Q30 proportion was high, indicating that the sequencing bases had a high-quality value of ≥30. Abnormal anti-Brucella antibodies and antibody titers further verified the mNGS results. However, mNGS can be influenced by various factors, emphasizing the need for strict adherence to specimen collection guidelines to minimize contamination risk.[20]

The gold standard for meningitis diagnosis is a positive culture. In this study, Brucella was not cultured from CSF but was detected by mNGS, with varying sequences. Studies have demonstrated that CSF mNGS is more sensitive than serological tests and culture for diagnosing neurobrucellosis.[21–23] While neurobrucellosis in children is rare,[24] the first pediatric case was reported in the United States in 2017.[25] Expanding sample sizes and increasing sequencing depth are necessary for further research.

It is critical to differentiate neurobrucellosis from brucellosis, as neurobrucellosis requires longer treatment, typically involving a triple combination of ceftriaxone, rifampicin, doxycycline and cotrimoxazole, along with follow-up. Cases 2 and 3 were diagnosed and treated promptly, while Case 1, presenting with cerebral infarction, experienced delayed treatment. Effective rehabilitation and close follow-up are essential for patient recovery.

Accurate and timely diagnosis is crucial. In this study, mNGS enabled the diagnosis of CNS infections. It is recommended that children in endemic regions with suspected neurobrucellosis undergo mNGS as soon as possible.

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