Neonatal *Ureaplasma parvum* meningitis: a case report and literature review

Qiu Wang[1#], Kai Wang[1#], Yuanbo Zhang[1], Chaosheng Lu[1], Yana Yan[1], Xiaoxia Huang[1], Jing Zhou[2], Lijiang Chen[3], Dan Wang[1]

[1]Department of Pediatrics, [2]Department of Obstetrics, [3]Clinical Laboratory, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

[#]These authors contributed equally to this work.

Correspondence to: Dan Wang, MD. Department of Pediatrics, The First Affiliated Hospital of Wenzhou Medical University, No. 2 Fuxue Road, Wenzhou 325000, China. Email: wd2014@126.com.

Abstract: *Ureaplasma parvum* (*U. parvum*) is common commensal in the female genitourinary tract. Despite *U. parvum* has been associated with chorioamnionitis, abortion, prematurity and perinatal complications, the invasive central nervous system (CNS) infection is rare in neonates. Diagnosis of *U. parvum* meningitis can be difficult for the atypical presentations and sterile cultures by conventional methods. Metagenomic next-generation sequencing (mNGS) could identify a broad range of human pathogens in a target-independent manner. Here, we performed mNGS to search for the infectious etiology in a term infant presenting with fever and seizure. *U. parvum* genome was identified by mNGS and further confirmed by PCR in the same cerebrospinal fluid (CSF) sample. As the quick and timely diagnosis, the baby was successfully treated with erythromycin for 4 weeks without complication. The clinical follow-up has showed that the physical and mental development are normal. In conclusion, mNGS may a promising diagnostic technology for *U. parvum* meningitis. As mNGS is able to identify diverse microbes in a single run, it could be a useful strategy to detection the clinical causative pathogens with atypical features in neonates.

Keywords: Neonate; meningitis; metagenomic next-generation sequencing (mNGS); *Ureaplasma parvum* (*U. parvum*); case report

Introduction

Ureaplasma species (spp.), including *Ureaplasma urealyticum* (*U. urealyticum*) and *Ureaplasma parvum* (*U. parvum*), are the smallest free-living organisms, lacking cell-wall, colonizers of the mucosal epithelial cells of genitourinary tract (1). They have been associated with a serial of obstetrical complications, including chorioamnionitis, premature rupture of membranes, prematurity, miscarriage, still birth, and perinatal morbidity and mortality (1,2). Since the first case report of *U. urealyticum* meningitis in newborn in 1986, several researches have been indicated that *U. urealyticum* could be a causative pathogen of invasive disorders, such

as pneumonia, bacteremia and central nervous system (CNS) infection (2,3). However, the invasive infection in neonates with *U. parvum* has been rarely presented. The diagnosis of *U. parvum* meningitis is complicated by the atypical manifestations and the inherent biologic properties of this organism. *U. parvum* cannot be demonstrated on Gram staining and may not grow in conventional culture media. Unlike traditional assessment for specific pathogen, metagenomic next-generation sequencing (mNGS) screens for nearly all potential CNS infections and can identify novel or unexpected microbes. mNGS is being used increasingly for the clinical diagnosis of CNS infection in adults. Here, we present a rare case of *U. parvum* meningitis
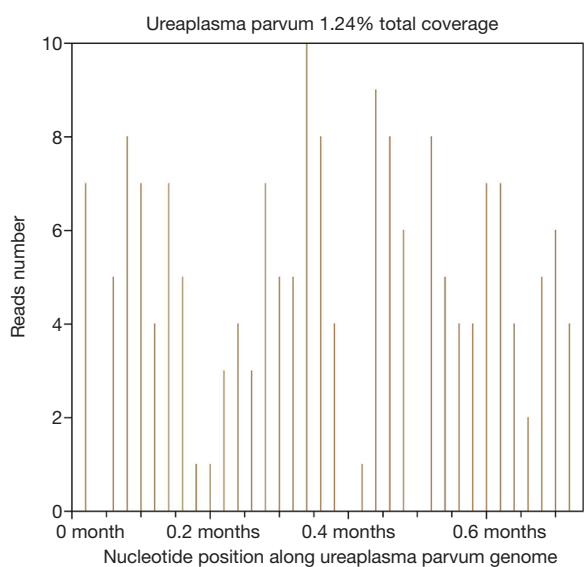
Figure 1 Sequence reads mapped to *U. parvum* by mNGS data. A total of 188 reads mapped to *U. parvum* in the reference database, corresponding to a total coverage of 1.24%.

diagnosed by mNGS in a neonate. As the timely diagnosis, the baby was successfully treated with erythromycin without complication. This report aims to discuss the characteristics of neonatal *U. parvum* meningiti*s*, including clinical features, new methods for microbial diagnosis, pathogenicity, and the challenges of appropriate treatment. We present the following case in accordance with the CARE Guideline (4).

Case presentation

A 11-day-old male infant (birth weight 3,390 g) was admitted to hospital for a sudden onset of fever (38.7 ℃) and floppy. The baby was born by spontaneous vaginal delivery to a 41-year-old gravida 0 para 0 mother at 40 weeks of gestation. He did not have surface anomalies and was vigorous at birth with Apgar scores of 8 at 1 min and 9 at 5 min. Maternal pregnancy was complicated by gestational diabetes mellitus and premature rupture of the membranes for 40 hours. The Blood analysis revealed leukocytosis (15,740/mm^3), and elevated C-reactive protein (10.4 mg/L) with normal level of procalcitonin (0.04 ng/mL). Cerebrospinal fluid (CSF) assessment showed leukocytosis (410 WBC/mm^3 with 55% of neutrophils), elevated protein level (2.0 g/L), and reduced glucose (<1.11 mmol/L). Combined treatments of

intravenous oxacillin (50 mg/kg/dose, q8h) and sulperazon (50 mg/kg/dose, q8h) were started for bacterial meningitis. No microorganism was detected on Gram staining or cultures of blood and CSF samples. On the day 4 of hospitalization, the infant presented local seizures and his anterior frontal became full and bulging. A cranial magnetic resonance image (MRI) identified ventriculitis and mild dilatation of right lateral ventricle, but no abscess or bleeding. A new CSF was analyzed and results showed 880 WBC/mm^3 (48% of neutrophils), 2.6 g/L protein, and <1.11 mmol/L glucose. Antibiotic therapy was modified to intravenously administered vancomycin (15 mg/kg/dose, q8h) and meropenem (30 mg/kg/dose, q8h); and phenobarbital was added to suppress seizure. Again, bacteriological cultures of blood and CSF still negative. After 7 days treatment of vancomycin and meropenem, the infant had a sustained fever of 38.0 ℃ and somnolent, a third CSF was collected, which showed 205 WBC/mm^3 (44% of neutrophils), protein at 1.9 mg/L, and glucose at 1.9 mmol/L. Given CSF culture failed to reveal a pathogen, a CSF sample was collected and sent for the unbiased mNGS (BGI, Shenzhen, China), with a classification reference databases downloaded from NCBI (ftp://ftp.ncbi.nlm.ni-h.gv/genomes/) containing 1,424 whole genome sequence of viral taxa, 2,406 bacterial genomes or scaffolds, 199 fungi related to human infection and 135 parasites associated with human diseases (5). Within 72 hours, the mNGS produced 36.1 million reads and detected 188 reads of *U. parvum*, covering 1.24% (9,306/751,719) of the *U. parvum* genome (*Figure 1*). To further verify the presence of this pathogen, a specific PCR analysis was conducted with the same CSF sample and the PCR product was assessed by Sanger sequencing. The PCR primers were 5'-CATTGATGTTGCACAAGGAGAAA-3' and 5'-TTAGCACCAACATAAGGAGCTAAATC-3', which target a *U. parvum* specific region of 16S rRNA gene. Based on these results, the patient was diagnosed of *U. parvum* meningitis and was administered with intravenous erythromycin (10 mg/kg/dose, q6h); other antibiotics were weaned. On day 3 and 7 of erythromycin treatment, the fever subsided and CSF test recovered, respectively; and his condition continued favorably. After the 4 weeks course of erythromycin, the repeated CSF was without biochemical abnormalities and baby was discharged home. The timeline of this case was shown in *Figure 2*. The infant developed well without abnormal neurological signs during the follow-up until 18 months old. His head MRI at the age of 6 months showed the resolution of ventriculitis and hydrocephalus.
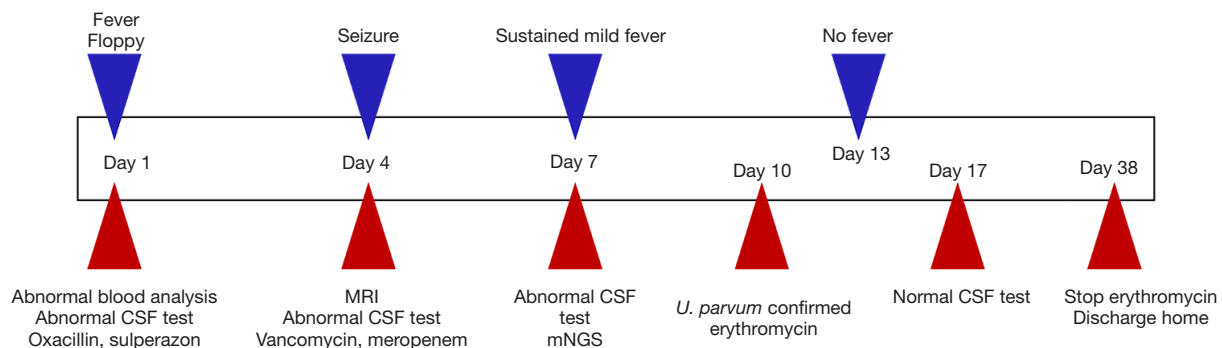
Figure 2 The timeline of this case, including clinical presentations, tests and treatments.

Table 1 Characteristics of published cases with *Ureaplasma parvum* meningitis

No.	Gender	Onset time	Presentation	Identification	Treatment	Complication	Others	Ref.
1	Female	18 days after birth	Fever, cardiovascular instability and apnea	PCR	ERY + CIP × 3 weeks	Grade III IVH and HCP	Preterm, fetoscopic surgery at 21 gestational weeks	(5)
2	Female	4 weeks after birth	HCP and developmental delay	PCR	CHL × 3 weeks	HCP and developmental delay	Preterm, maternal cervical insufficiency and cerclage	(6)
3	Unknown	10 days after birth	Fever, rhinitis, conjunctivitis and focal seizure	PCR and culture	CIP ×7 weeks + THI ×3 weeks	HCP	Term	(7)
4	Male	16 days after surgery	Fever, headache, photophobia and meningeal irritation	PCR	LVFX ×3 weeks	None	Adult, craniopharyngioma ablation	(8)

IVH, intraventricular hemorrhage; HCP, hydrocephalus; ERY, erythromycin; CIP, ciprofloxacin; CHL, chloramphenicol; THI, thiophenicol; LVFX, levoflfloxacin.

Discussion

Here, we describe a rare case of neonatal meningitis resulting from *U. parvum* infection. Like the most previous cases of *U. parvum* meningitis (6-9), the common cultures of blood and CSF were negative. But after the pathogen was identified by mNGS and confirmed by PCR, the baby was successfully treated with erythromycin.

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Despite *U. parvum* has been consistently associated

with histological chorioamnionitis, abortion, stillbirth, preterm birth and perinatal complications (1,2), the invasive infection is rare. According to the literature, there are four cases of *U. parvum* meningitis have been previously reported (*Table 1*) (6-9). One is in an adult after surgical ablation of a craniopharyngioma (9). Other three cases are in neonates (two prematurity and one term) (6-8). In all reported neonatal cases, no other pathogen was found despite extensive microbiological diagnostics. Infection with *U. parvum* in neonates has been documented to be late onset meningitis and has variable clinical presentation including fever, apnea, cardiovascular instability, rhinitis, conjunctivitis, seizure and hydrocephalus. In this case, the full-term boy also had manifestations of fever, floppy and seizure. The results of initial CSF analysis might with the predominance of leukocytes or lymphocytes and the glucose

level could be undetectable or normal, suggesting bacterial or viral meningitis. Similarly, the CSF was presented with pleocytosis and decreased glucose level in our case. Thus, the CSF profile of *U. parvum* meningitis may be various and atypical. After the specific treatment of *U. parvum*, the CSF profile has been reported to gradually normalize, paralleled by a continuous clinical improvement. Development of hydrocephalus with or without of hemorrhage also has been reported in all neonatal cases, either at initial signs or in the clinical course. And the long-term outcome of neonatal *U. parvum* meningitis ranges from complete resolution to progressive internal hydrocephalus or neurodevelopmental impairment (6,8). In accordance to the previous *U. parvum* meningitis, the hydrocephalus gradually resolved and the baby boy develops appropriately in this case. Only one previous case which manifested with sustained meningitis and progressive hydrocephalus had poor neurological outcome (6,8), implying that continuous inflammation is related to the adverse outcome. Thus, early diagnosis and initiation of appropriate treatment are necessary to improving the prognosis.

Although *Ureaplasma* normally exist in a state of adherence to mucosal epithelial cells, they can disseminate to other sites and cause invasive infection when there is a disruption of the mucosa and/or an underlying defect in host defenses, such as in the developing fetus or newborn. Their pathogenicity has been imputed to inflammatory response by modulating the immune system in a complex way, not only promoting pro-inflammation, but also inhibit immune response (1,10). The balance between pro-inflammatory cytokines (e.g., tumor necrosis factor-α, interleukin (IL)-1β, IL-2, IL-6, IL-8) and anti-inflammatory cytokines (e.g., IL-10) is disturbed by *Ureaplasma* infection (1). In addition, they could also affect innate host immune by downregulating cell defense genes (e.g., antimicrobial peptides), decomposing the host cell membrane via phospholipase, and exerting cytotoxicity through ammonia accumulation or PH-shift via hydrolyzing urea (11,12).

In the previous *U. parvum* meningitis cases, the pathogen in CSF was demonstrated by PCR in three cases and by the combination with PCR and culture in on case. *Ureaplasma* are the smallest free-living organisms. They cannot be visualized by Gram stain since the lacking of cell wall (13). Given they are not surviving in standard culture media, in order to harvest these organisms, clinical specimens should be inoculated immediately onto specialized culture media before they are allowed to dry (7,13). And culture detection take long time intervals and positive rate can be as low as

40% (14). On account of the complex growth requirements and specific culture condition, the organism might avoid detection. Broad range bacterial PCR is another method for the *Ureaplasma* identification and superior to traditional culture methods. But this hypothesis-driven molecular testing may miss the pathogen when there is no consideration about the specifically targeted organisms or when the used primers contain mismatches to the microbial strain involved, which decreases the sensitivity of detection. Above options for diagnosing infections caused by *Ureaplasma* are limited due to the shortcomings of both culture and PCR-based techniques. Thus, microbial diagnostics on *Ureaplasma* may be delayed by the difficulties in pathological detection. Here, we found *U. parvum* in CSF by mNGS analysis. mNGS, a high-throughput or massively parallel sequencing method, is beneficial for diagnosis in patients with suspected infectious but negative results for conventional assessments (5). It could apply for hypothesis-free and universal pathogen detection, promising to improve infectious disease diagnostics, especially in immunocompromised neonates or critically ill patients. This novel technique will enable timely diagnosis and treatment of invasive *U. parvum* infection and is expected to result in excellent outcomes, as presented in this case. However, mNGS method also has inherent disadvantages, including sequencing human host gene background, suffering from the contamination with environmental species, and the expensive cost.

The treatment of *invasive Ureaplasma infection* in neonates is particularly challenging. *Previous cases of U. parvum* meningitis shows several therapeutic chemicals (*Table 1*). Three reports in neonates used different antibiotic regimes: two preterm infants were treated by chloramphenicol and the combination of erythromycin and ciprofloxacin, respectively; the other term baby was administered with ciprofloxacin and thiophenicol. Considering that *Ureaplasma a*re free of cell wall, the antibiotics targeting protein or DNA synthesis may effective, such as macrolides, tetracyclines, clindamycin, chloramphenicol and quinolones (14). However, the potential side effects of above drugs have been taken into consideration when used in the newborn. Tetracyclines can induce permanent dental discolorations or skeletal abnormalities; chloramphenicol may cause "gray baby syndrome" or blood dyscrasias; and fluoroquinolones have arthropathogenic effects. Thus, these drugs are generally avoided in neonates due to concerned toxicities. Macrolide antibiotics are usually used in the *Ureaplasma* treatment. Here, we firstly treated the baby with erythromycin. Erythromycin is not suggested for the treatment of

Ureaplasma meningitis due to its poor liquor penetrance (14). But some cases have been successfully treated with erythromycin (15), which may be explained by the increase of blood-brain barrier permeability when meningitis is occurring. In addition, the appropriate duration of antibiotic therapy for *Ureaplasma* meningitis is not well understood. We choose erythromycin for 4 weeks, as is general practice in the treatment of other types of bacterial meningitis in neonates. In other reports, the treatment duration may range from 2 to 6 weeks in infants (16).

In conclusion, neonatal *Ureaplasma* meningitis might be more prevalent than previously suspected. Its presentations and CSF changes may be not typical. Any infant suffering from internal hydrocephalus, with evidence of congenital infection, or with CSF pleocytosis, should be recommend to have CSF microbial diagnostics on *Ureaplasm*a. Early diagnosis and initiation of appropriate treatment could prevent the adverse outcome. Identification of *U. parvum* can be difficult for it may escape common detection due to complex culture media and condition. mNGS can identify diverse microbes in one run, it could be a promising strategy to diagnosis *U. parvum* meningitis or detect the causative pathogens with atypical clinical features in neonates.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tp.2020.02.04). KW serves as the unpaid section editor of *Translational Pediatrics* from Oct 2019 to Dec 2020. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the ethical review committee of The First Hospital Affiliated to Wenzhou Medical University. Written informed consent was obtained from the patient for publication of this Case report and any accompanying images.

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