Neonatal *Pasteurella multocida* subsp. *septica* Meningitis Traced to Household Cats: Molecular Linkage Analysis Using Repetitive-Sequence-Based PCR

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***Pasteurella multocida* is a rare cause of neonatal bacterial meningitis. We describe such a case and verify two household cats as the source of infection using repetitive-element PCR (rep-PCR) molecular fingerprinting.**

CASE REPORT

A 17-day-old boy was seen in the emergency department after a 2-day history of worsening fussiness, irritability, fever, and bilateral eye crusting of 7 days' duration. Upon presentation, his rectal temperature was 39.7°C, and his heart and respiratory rates were 149 beats/min and 44 breaths/min, respectively. Laboratory studies showed the following: hemoglobin, 15.7 g/dl; hematocrit, 44.6%; white blood cell (WBC) count, 18.2 billion/liter (neutrophils, 63%; lymphocytes, 23%; monocytes, 14%); platelet count, 379 × 10⁹/liter; Na, 129 mmol/liter; K, 5.4 mmol/liter; chloride, 95 mmol/liter; CO₂, 22 mmol/liter; blood urea nitrogen (BUN), 12 mg/dl; creatinine, <0.1 mg/dl; albumin, 4.0 g/dl; alkaline phosphatase, 648 U/liter; total bilirubin, 3.1 mg/dl; aspartate transaminase (AST), 61 U/liter; and alanine aminotransferase (ALT), 50 U/liter. Cerebrospinal fluid (CSF) studies showed 1,500 red blood cells/μl, 20,822 WBCs/μl (neutrophils, 73%; mononuclear cells, 27%), 7 mg/dl glucose, and 148 mg/dl protein. Gram stain showed many polymorphonuclear leukocytes, moderate mononuclear cells, and rare Gram-negative bacilli. Bacterial antigen was negative for *Haemophilus influenzae* type b, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Neisseria meningitidis* (A, B/E, C, Y, and W135), and *Escherichia coli* K1. Cefotaxime, ampicillin, and acyclovir (one dose) were empirically started. He was transferred to the pediatric intensive care unit (PICU).

On examination, the infant had a patent, slightly full anterior fontanelle and a right-sided middle ear effusion with mild tympanic membrane injection. He was tachycardic with a grade I-II/VI systolic murmur. There was full passive range of motion of all joints; none were red, warm, or swollen. Kernig and Brudzinski signs were negative. Moro and stepping reflexes were present. There was no neck stiffness, and he had good motor tone. Ampicillin was discontinued, and he was treated with cefotaxime (200 mg/kg body weight/day every 8 h) and gentamicin (2.5 mg/kg/dose every 8 h). An echocardiogram revealed physiologic peripheral pulmonic stenosis and a patent foramen ovale. The xTAG respiratory viral panel (Luminex, Austin, TX) was negative. By history, the neonate was born at term to a 35-year-old GI (primigravida) mother who had no illnesses during her pregnancy and received no antibiotics; she developed hypertension late in pregnancy and was induced at 39 weeks' gestation. She delivered by cesarean section 10.5 h after rupture of membranes. She was

screened during pregnancy for HIV-1 antibodies, hepatitis B surface antigen, VDRL test, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group B *Streptococcus* anogenital carriage; all tests were negative. The neonate lived with his mother, father, and maternal grandmother; a 10-year-old paternal half-brother stayed with them every other weekend. The family had one dog and two cats that were from the same litter. The family pets were allowed around the neonate, but there was no known traumatic contact (i.e., bites or scratches). *Pasteurella multocida* was recovered from blood and CSF of the neonate. Gentamicin was discontinued; intravenous cefotaxime was given for a total of 14 days. He developed jaundice that did not require phototherapy. Two days after admission to the PICU, repeat lumbar puncture showed the following: 0 red blood cells/μl, 180 white blood cells/μl (neutrophils, 56%; lymphocytes, 11%; monocytes, 33%), 28 mg/dl glucose, and 127 mg/dl protein; Gram stain showed many polymorphonuclear leukocytes and mononuclear cells and no bacteria. Repeat CSF culture was negative for bacterial growth. Gadolinium magnetic resonance imaging (MRI) of the head showed diffuse enhancement of the leptomeninges and the ependymal lining of the occipital horns. He recovered without hearing loss or neurodevelopmental delay. He received a dose of the hepatitis B vaccine prior to discharge.

Cerebrospinal fluid (CSF) and blood were obtained from the patient using a sterile technique and subjected to conventional plate-based (sheep blood and MacConkey agar, 35 to 37°C incubation) and automated fluid-based (BacTAlert; bioMérieux) cultures, respectively. Oropharyngeal swabs obtained from the family dog and gingival swabs from both family cats were placed into

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FIG 1 Repetitive-element PCR results (in duplicate) for *Pasteurella multocida* subsp. *septica* isolates obtained from the neonate (CSF), both family cats, and archived clinical isolates using the universal bacterial strain typing kit and universal bacterial consensus primers.

Amies transport medium and subjected to conventional plate-based culture as previously described. *P. multocida* was recovered from the neonate's blood and CSF as well as both cats, with microbial identification probability scores of >96% and >99% for the manual (RapID NH, Remel, Lenexa, KS) and automated (MicroScan, Siemens, Washington, DC; Vitek II, bioMérieux, Durham, NC) biochemical methods, respectively. *P. multocida* was not isolated from the dog. Repetitive-element PCR (rep-PCR) (DiversiLab; bioMérieux, Durham, NC) was utilized to delineate the source of neonatal infection. Using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Solana Beach, CA), DNA was extracted from six *P. multocida* isolates from the infant ($n = 1$; CSF), cats ($n = 2$), and archived clinical isolates ($n = 3$). Archived isolates were obtained from the clinical microbiology laboratories of William Beaumont Hospital ($n = 1$) and Texas Children's Hospital ($n = 2$). These isolates were cultivated from localized skin infections of three different patients and identified as previously described. rep-PCR was performed in duplicate with the general bacterial fingerprinting kit (411007; bioMérieux, Durham, NC) utilizing both general bacterial consensus primers and *Pasteurella*-specific primers REP1R-IDt (5'-NNN NCG NCG NCA TCN GGC-3') and REP2-IDt (5'-NCG NCT TAT CNG GCC TAC-3') as previously described (1). Amplified products were loaded onto the DNA LabChip (Agilent Technologies, Palo Alto, CA) and processed on the Agilent 2100 Bioanalyzer to generate molecular profiles via microfluidics-based electrophoresis. The resulting molecular profiles were automatically transferred to the DiversiLab 3.1 software (bioMérieux, Durham, NC) for analysis using Pearson's correlation coefficient and unweighted pair group with arithmetic mean analysis (UPGMA) to generate dendrograms, scatterplots, and similarity matrices. rep-PCR showed that *P. multocida* isolates from the infant and both family cats were indistinguishable. In contrast, the three archived *P. multocida* isolates were distinct from each other as well as from the infant and cat isolates. (Fig. 1 and 2) All isolates

were confirmed as *P. multocida* subsp. *septica* by full 16S rRNA bidirectional Sanger sequencing.

P. multocida is a Gram-negative coccobacillus that normally inhabits the oral microbiome of many domestic animals and can be phylogenetically subdivided into three subspecies: *P. multocida* subsp. *multocida*, *P. multocida* subsp. *gallicida*, and *P. multocida* subsp. *septica* (2, 3). *P. multocida* subsp. *septica* is most commonly isolated from cats (3). *P. multocida* is commonly implicated in localized infections (i.e., acute cellulitis and lymphadenitis) following the bite of household pets, especially cats or dogs. Other routes of acquisition include nontraumatic exposure (i.e., licking or scratching from household pets) and vertical transmission (2). *P. multocida* is rarely implicated as a cause of meningitis; about 25 cases have been reported in newborns (≤ 30 days of age) since 1953. The clinical presentation, laboratory diagnosis, treatment, outcome, and potential risk factors for neonatal *P. multocida* meningitis are reviewed elsewhere (2, 4). In the vast majority of these reported cases, studies linking the organism to an animal source were largely established using phenotypic methods (i.e., serologic typing of capsular or somatic antigens or comparison of antibiogram patterns) that lack sufficient discriminatory power. Definitive linkage analysis is traditionally performed with pulsed-field gel electrophoresis (PFGE), ribotyping, or restriction endonuclease analysis; however, these fingerprinting methods are time-consuming, labor-intensive, and not commonly utilized. Such disadvantages likely explain the paucity of peer-reviewed data using these methods to definitively establish the source of infection in reported cases of neonatal *P. multocida* meningitis (5–9). Repetitive sequence-based PCR (rep-PCR) is another fingerprinting method that uses oligonucleotide consensus primers targeting noncoding genetic elements scattered throughout the bacterial genome as a foundation for PCR-based amplification of interven-

FIG 2 Repetitive-element PCR results (in duplicate) for *Pasteurella multocida* subsp. *septica* isolates obtained from the neonate (CSF), both family cats, and archived clinical isolates using the universal bacterial strain typing kit and *Pasteurella*-specific primers.

ing coding sequences. The amplified products (size range, 200 to 3,000 bp) are electrophoretically separated to generate a unique bacterial fingerprint. Computer software analyzes the sizes and intensities of different bacterial fingerprints to determine a genetic relatedness score. This technology has been used for numerous applications in both the research and clinical arenas and has been shown to compare favorably with traditional fingerprinting methods to accurately discriminate between different strains of *P. multocida* isolated from avian and swine sources (1, 9, 10). As presented herein, we have demonstrated the excellent discriminatory power of rep-PCR to clearly delineate the source of neonatal *P. multocida* meningitis. To our knowledge, this is the first report applying rep-PCR technology in this capacity and more specifically the ability to distinguish between *P. multocida* isolates at the subspecies level. Several molecular fingerprinting methods are commercially available. These valuable tools are crucial for epidemiologic investigations and enhancing our ability to understand transmission routes for infectious agents.

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