Gestational Psittacosis Resulting in Neonatal Death Identified by Next-Generation RNA Sequencing of Postmortem, Formalin-Fixed Lung Tissue

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Psittacosis is a rare zoonosis that can cause severe disease and adverse outcomes during pregnancy. We identified a previously elusive case of psittacosis causing premature delivery and infant death by next-generation RNA sequencing of postmortem tissues. Hypothesis-free pathogen detection in postmortem specimens can increase the yield of epidemiologic and cause-of-death studies.

Keywords. Chlamydia psittaci; gestational psittacosis; metagenomics; neonatal pneumonia.

Chlamydia psittaci is an obligate intracellular bacterium that infects a wide range of bird species and can cause zoonotic infections in human (psittacosis). Sequencing of the major outer-membrane protein (ompA) gene allows differentiation of 10 genotypes [1]. Humans typically acquire psittacosis through inhalation of aerosolized bird droppings, mammalian feces, plumage, or close contact with infected birds or mammals. Less commonly, C. psittaci can be transmitted from human to human [2, 3]. Although often asymptomatic or mild, typical psittacosis is characterized by fever, headache, malaise, and atypical pneumonia [4]. Infections of the liver, spleen, central nervous system, and other organs are less common. In the United States, psittacosis is rare, with only 92 reported cases between 2005 and 2015 [5]. However, the true incidence is likely higher given unspecific symptoms and limited availability of diagnostic tests [6, 7]. Tetracyclines are the preferred treatment, and fatal cases are rare. However, infections during pregnancy can result in maternal pneumonia and sepsis, placental involvement, and premature delivery or miscarriage. Gestational psittacosis is rare, with <5 reported cases in the United States and <20 reported cases globally [8–12].

Here we report a case of gestational psittacosis that caused unspecific febrile disease in the mother and that resulted in premature labor at 21 weeks followed by neonatal death that was diagnosed retrospectively by hypothesis-free, metagenomic next-generation sequencing (NGS) of banked formalin-fixed neonatal tissues.

CASE REPORT

In October of 2012, a 32-year-old pregnant female (13 weeks’ gestation, G2P0010) was seen at an urgent care center for fever, neck pain, headache, sinus pressure, nausea, and fatigue and was prescribed a 10-day course of cefuroxime. Three weeks later, she was admitted to a local hospital with persistent fever. Physical examination demonstrated hepatosplenomegaly, and laboratory testing revealed normal white blood cell count with left shift and toxic changes, normocytic, normochromic anemia, thrombocytopenia, and positive anticardiolipin IgM antibodies (lupus anticoagulant, anticardiolipin IgG, beta-2 glycoprotein 1 IgG and IgM antibodies were negative; all antiphospholipid antibody tests were negative 2 months later). No serologic studies for Chlamydia psittaci were performed. A bone marrow biopsy with flow cytometric studies demonstrated left-shifted erythroid and myeloid hematopoiesis with an increased number of morphologically normal megakaryocytes and increased expression of CD64 on myeloid cells. After extensive, unrevealing workup, she was diagnosed with undifferentiated connective tissue disorder and treated with hydroxychloroquine and prednisone. Four weeks after discharge, she presented to the emergency department with mild vaginal bleeding and suspected preterm labor, but she was discharged after improvement. Two weeks later, she was admitted again for premature preterm rupture of membranes at 21 weeks’ gestation and spontaneously delivered a 320-g infant with APGAR scores of 1 and 0 at 1 and 5 minutes. Resuscitation was not attempted due to immaturity and severe low birth weight. At a follow-up visit 1 month after delivery, the patient was afebrile and without signs or symptoms of connective tissue disorder.

Postmortem examination demonstrated a premature, small for gestational age infant with evidence of intra-amniotic infection and congenital pneumonia with marked neutrophilic infiltration of distal airspaces bilaterally (Figure 1A, B). Gomori methenamine-silver nitrate stain of lung and placental tissue sections for fungi was negative. Complications of prematurity included mild cerebral hemorrhage in the germinal matrix and subarachnoid space. The placenta was malodorous with...
Figure 1. Histopathologic findings of postmortem infant lung (A, B) and placental tissues (C, D). A, The lung is appropriately developed for the gestational age and shows congenital pneumonia characterized by neutrophils within terminal bronchioles and distal airspaces. B, Higher magnification. C, The maternal inflammatory response is characterized by neutrophils within the fibrin layer under the chorionic plate (subchorionitis) and within the chorionic plate (chorioamnionitis). D, Fetal inflammatory response is uncommon in this early gestational age but is shown here with neutrophils in the wall of a chorionic plate vessel. E, Alignment of RNA sequencing data from postmortem, formalin-fixed, paraffin-embedded infant lung tissue resulted in 126-fold mean coverage of the *Chlamydia psittaci* genotype D reference sequence (CP003798) with only 1 mismatch across the entire gene. F, A phylogenetic tree of the 16S rRNA consensus sequence from the patient strain and reference strains for members of the *Chlamydia* group [15] shows close clustering with the genotype D reference strain (*Neochlamydia hartmannellae* was used as the outgroup). G, Real-time polymerase chain reaction (PCR) detected *Chlamydia psittaci* DNA in a range of fetal, placental, and maternal tissues. The heatmap shows relative differences in threshold cycles between the tissue with the highest concentration (umbilical cord) and other PCR-positive tissues; negative results are shown in white. Abbreviation: BM, bone marrow.
multiple marginal and central infarcts and a large retroplacental hematoma. Histopathologic examination demonstrated acute chorioamnionitis (stage 3) (Figure 1C), mild funisitis (stage 1) (Figure 1D) with perivenous predominance, and large areas of maternal floor infarct with occasional intravascular thrombi. Cause of death was attributed to amniotic fluid infection syndrome leading to premature delivery.

Next-generation RNA sequencing (RNA-seq) was performed on banked, formalin-fixed, paraffin-embedded (FFPE) postmortem lung tissue from the infant (University of Utah Institutional Review Board No. 00093124). RNA was extracted (RNeasy FFPE Kit, Qiagen) after complete proteolytic digestion of FFPE tissue, sequencing libraries were prepared (KAPA Stranded RNA-Seq Kit with RiboErase, Roche without additional RNA fragmentation), and RNA was sequenced on a NextSeq500 instrument (Illumina) to a depth of ~10 million reads/sample. No other human RNA depletion methods were used. Postmortem FFPE lung tissues from 5 infants without evidence of pneumonia were used as controls. Hypothesis-free pathogen detection with Taxonomer [13] identified C. psittaci (Supplementary Figure 1). Abundance of human and microbial RNA is shown in Supplementary Figure 3. Results were confirmed by alignment to the genome of the C. psittaci type strain (Geneious, v. 8.1, Biomatters) showing a mean of 126- and 128-fold coverage and 99.5% and 99.8% sequence identity with the small (16S, GenBank accession NR_036864) and large subunit (23S, NR_103183) rRNA of the C. psittaci type strain, respectively. The most similar C. psittaci strain was strain NJ1 (CP0035798), with 99.9% and 100% sequence identity with the 16S (Figure 1E) and 23S rRNA genes (Supplementary Figure 3). Phylogenetic analyses demonstrated close clustering with genotype D of C. psittaci (Figure 1F; Supplementary Figure 2). Other organisms detected belonged to the genera Achromobacter and Delftia but were also found in FFPE lung tissue of 2 or more of the controls, suggesting that they were due to common reagent or process contamination (Supplementary Figure 4).

To confirm detection of C. psittaci and screen additional tissues, we tested RNA and DNA (extracted with the QiaAMP FFPE DNA Kit, Qiagen) from all available FFPE tissues by real-time polymerase chain reaction (PCR) targeting the C. psittaci ompA gene [14]. Results confirmed detection of C. psittaci in infant lung tissue, strong amplification in the placenta and fetal membranes, and lower levels of C. psittaci in additional tissues (Figure 1G). The maternal bone marrow showed late but reproducible amplification. Real-time PCR for C. abortus [14] was negative.

**DISCUSSION**

Hypothesis-free, NGS-based pathogen detection is a powerful tool for the diagnosis of unexpected, rare, and genetically diverse pathogens as well as those that are difficult to culture. Initially pioneered for outbreak investigations and pathogen discovery, NGS-based pathogen detection is now being rapidly adopted by diagnostic laboratories. Testing of banked, formalin-fixed tissues creates immense opportunities for the study of rare diseases but remains technically challenging due to fragmented DNA and RNA, the abundance of host nucleic acid competing with pathogen detection during next-generation sequencing (Supplementary Figure 2), and the potential for contaminants to be introduced during tissue processing and storage. We applied NGS-based RNA sequencing to detect a previously undiagnosed case of vertically transmitted psittacosis using banked FFPE tissues. Results were confirmed by PCR using infant, placental, and maternal tissues and are consistent with histopathologic findings in postmortem infant lung and placental tissues.

Psittacosis during pregnancy is rare but can cause substantial maternal and fetal morbidity and mortality [11]. Its low incidence—no cases of psittacosis have been reported in Utah since 2004 [5]—unspecific symptoms, and lack of routinely available tests pose diagnostic challenges. Absence of typical risk factors posed further challenges for a diagnosis in the present case: The mother had no known exposure to birds or farm animals, worked in an office environment, and the only known animal exposure was to a pet cat at home.

Our study highlights the power of NGS-based, hypothesis-free pathogen detection to detect previously missed pathogens in postmortem tissues. Wide availability of postmortem tissues makes this a promising approach for epidemiologic and cause-of-death studies.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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