

Diagnosis of a Familial Psittacosis Outbreak with Clinical Analysis and Metagenomic Next-Generation Sequencing Under COVID-19: A Case Series

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Purpose: To explore the clinical characteristics, diagnosis, and treatment of family outbreak of psittacosis and to improve the success rate of treatment.

Patients and Methods: The clinical characteristics, diagnosis, treatment, and outcome of family outbreak of psittacosis, which consists three patients, diagnosed by clinical analysis and metagenomic next-generation sequencing (mNGS) in our hospital were analyzed retrospectively.

Results: We report on three instances of clustered atypical pneumonia, which were caused by *Chlamydia psittaci* during the COVID-19 pandemic. All patients exhibited symptoms of fever and cough, while one patient also experienced gastrointestinal symptoms such as nausea, vomiting, and diarrhea. Laboratory tests indicated no significant increase in leukocytes and neutrophils, but a mild increase in C-reactive protein was observed in all three patients. Chest computed tomography (CT) scans revealed a consolidation shadow in a unilateral lung lobe in all three patients. Both patients were treated with empirical moxifloxacin, yielding unsatisfactory outcomes. mNGS was conducted on sputum samples from one adult patient, revealing the presence of *Chlamydia psittaci*. Additional doxycycline was prescribed immediately, and then the patients' temperatures were stabilized, and the lesion in chest CT was absorbed. The pediatric patient exhibited less severe symptoms compared to the adult patients and exhibited a favorable response to azithromycin administration.

Conclusion: This study reports a cluster of a family outbreak of atypical pneumonia caused by *C. psittaci* in China. The occurrence of a family outbreak during the COVID-19 pandemic may be attributed to familial aggregation resulting from the epidemic. The three cases reported in this study did not experience severe complications, which can be attributed to the prompt medical intervention and swift diagnosis. This finding implies the need to enhance patients' awareness and vigilance towards their health. Additionally, mNGS emerges as a valuable technique for accurately identifying pathogens causing pulmonary infections.

Keywords: psittacosis, outbreak, COVID-19, metagenomic next-generation sequencing, pneumonia

Introduction

Chlamydia psittaci is an intracellular bacterium of Chlamydiaceae family.¹ Psittacosis, a zoonotic disease caused by *C. psittaci*, primarily affects individuals with close contact with birds, particularly poultry. The disease typically manifests in humans following exposure to infected poultry or their excreta and has an incubation period of 1–4 weeks. Psittacosis is usually sporadic,^{2,3} but human-to-human transmission has also been reported.^{4,5} There were a few reports of human psittacosis outbreaks in European countries, United States, and Australia etc.^{6–10} However, there were fewer reports on the clustered onset of *C. psittaci* in China.¹¹ The course of *C. psittaci* can vary from acute to

chronic, with clinical presentations ranging from asymptomatic to severe pneumonia. Additionally, patients may have multiple extrapulmonary complications, including acute kidney injury, rhabdomyolysis, and meningitis,^{12,13} which can be life-threatening if not managed promptly. However, rapid diagnosis of psittacosis in the early disease stages is difficult because of the similarity of the clinical presentation with other common community-acquired pneumonia.

Isolating and cultivating *C. psittaci* from sputum or alveolar lavage fluid are crucial diagnostic steps. However, culturing *C. psittaci* is a time-intensive endeavor and necessitates highly graded biosafety facilities and serological outcomes in patients are typically nonspecific, rendering precise diagnosis challenging. Metagenomic next-generation sequencing (mNGS) is a high-throughput sequencing technique that enables direct comparison of DNA/RNA in extracted clinical samples with a pathogen database. This method exhibits the ability to efficiently and objectively identify uncommon pathogenic microorganisms in clinical specimens, thereby presenting substantial potential for comprehensive infection diagnosis, particularly in instances of severe pneumonia.¹⁴

In this study, we present a comprehensive analysis of the clinical manifestations observed in a familial outbreak of psittacosis during the COVID-19 pandemic. The occurrence of family outbreaks during the COVID-19 pandemic may be associated with the clustering of cases within families as a result of the epidemic. The diagnosis of psittacosis was confirmed using mNGS, highlighting the utility of this technique in accurately identifying pulmonary pathogens causing infections.

Case Presentation

Patient 1

A 47-year-old male presented to the emergency department with fever, cough, dyspnea, and diarrhea. Notably, the patient had received a COVID-19 vaccination and reported owning a parrot. Laboratory findings indicated elevated levels of inflammatory markers, with a high-sensitive C-reactive protein (hsCRP) value of 33.4 mg/L (reference value <8 mg/L), but no significant changes in leukocyte or neutrophil counts (Table 1). Additionally, a chest computer tomography (CT) scan showed a scattered infiltrative patchy in the lower lobe of the right lung (Figure 1A). Following two days of empirical moxifloxacin treatment, hsCRP increased, and a repeat chest CT scan indicated significant expansion of the lesion (Figure 1B). His pCO₂ levels also rose, and his pO₂ levels, oxygen saturation, and oxyhemoglobin fraction significantly decreased. Considering the patient's history of exposure to parrots, he was admitted to the hospital for atypical pneumonia and underwent mNGS of the sputum. 51 specific *C. psittaci* sequences that covered 0.57% of the

Table 1 Clinical Characteristics and Laboratory Data of Three Patients

Items	Patient 1	Patient 2	Patient 3
Sex, age (years)	47, male	40, female	7, male
History of bird contact	Parrot	Parrot	Parrot
Underlying diseases	Hypertension, Diabetes, and renal calculi	None	None
Symptoms	Fever, cough, diarrhea	Fever, cough, fatigue	Fever, cough
Temperature (°C)	38.2	38.2	38.6
SpO ₂ (%)	96	97	98
WBC (normal: 4–10×10 ⁹ /L)	7.01	8.92	8.4
Neutrophil percentage (normal: 50–75%)	73.8	77.0	60.2
Monocytes percentage (normal 3–8%)	7.7	6.1	6.6
Lymphocytes percentage (normal 20–40%)	17.7	17.5	31.6
hsCRP (normal <8.0mg/L)	33.4	25.5	9.0
PCT (normal <0.5 ng/mL)	0.11	<0.072	<0.072

Abbreviations: WBC, white blood cell; hsCRP, high-sensitivity C-reactive protein; PCT, procalcitonin.

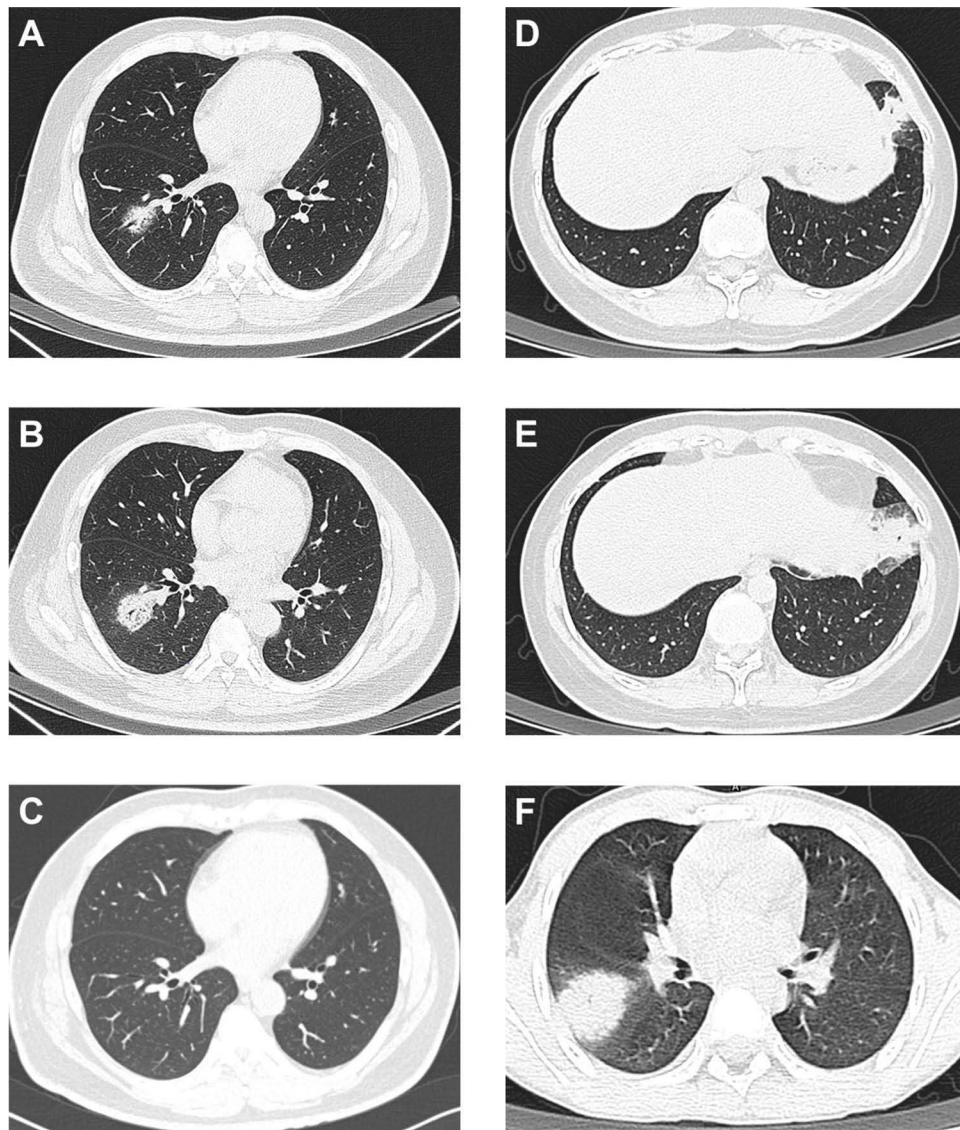


Figure 1 Chest CT images of *Chlamydia psittaci* pneumonia. (A–C) Chest computed tomography of patient 1 prior to treatment, after administration of empirical moxifloxacin and after administration of doxycycline. (D and E) Chest computed tomography of patient 2 before treatment and after administration of empirical moxifloxacin. (F) Chest computed tomography of patient 3 before treatment.

total *C. psittaci* genome were detected by mNGS-DNA, and 3 specific *C. psittaci* sequences that covered 0.03% of the total *C. psittaci* genome were detected by mNGS-RNA (Table 2). Subsequently, sanger sequencing was applied to further identify *C. psittaci* subtype based on its outer membrane protein A gene (*ompA*) sequence. The following primers (Table 3) were used to amplify the *ompA* gene from the DNA nucleic acid extracted from sputum. After amplification (Figure 2A) and sequencing, the *ompA* gene sequence in this case was analyzed using CLC Sequence Viewer 8, indicating that *ompA* gene sequence amplified from patient 1 was completely consistent with type A (Figure 2B). Consequently, the patient was prescribed two weeks of doxycycline (100 mg twice a day). A follow-up assessment six months later demonstrated complete resolution of pneumonia on repeat CT scans (Figure 1C). No sequencing or treatment was performed on the parrot.

Patient 2

A 40-year-old woman presented to the emergency department with fever, cough, nausea, and loss of appetite. She disclosed that she had received a COVID-19 vaccination and was the spouse of patient 1. Laboratory findings indicated elevated

Table 2 mNGS Results of the Psittacosis Pneumonia Cases

Detected Pathogens	Specific Reads (DNA)	Specific Reads (RNA)
<i>C. psittaci</i>	51	3
<i>P. intermedia</i>	20,419	–
<i>Gemella sanguinis</i>	12,987	–
<i>Streptococcus mitis</i>	7984	–
<i>Actinomyces</i> spp.	28,896	–
<i>Haemophilus parainfluenzae</i>	2297	–
<i>S. pneumoniae</i>	768	–
<i>H. influenzae</i>	127	–
<i>T. whipplei</i>	6	–

Note: “–” means pathogens were not detected.

Table 3 Amplification Primers of *ompA* Gene

Target Gene	PCR	Usage	Primer Name	Primer Sequence	Annealing Temperature	Product Length
<i>ompA</i>	General PCR	CPS Genotyping	CTU ompA-rev	5'-ATGAAAAAAGCTCTTGAAATCGG-3' 5'-TCCTTAGAATCTGAA TTGAGC-3'	50 50	1200bp

inflammatory markers, including a hsCPR of 25.5 mg/L (with a reference value of <8 mg/L) and a slightly increased proportion of neutrophils and monocytes (Table 1). A chest CT scan revealed a patchy consolidation in the left lower lobe (Figure 1D). She was prescribed empiric moxifloxacin, but her condition did not satisfactorily improve. Subsequent hsCRP test conducted two days later revealed a continued elevation, while a chest CT scan indicated the presence of a progressively expanding lesion (Figure 1E). She was then prescribed two weeks of doxycycline (100 mg twice a day) due to her husband's diagnosis of psittacosis through mNGS. A follow-up assessment six months later demonstrated the complete resolution of pneumonia on a CT scan. No sequencing or treatment was performed on the parrot.

Patient 3

A 7-year-old male patient presented to the emergency department with a fever. He had received the COVID-19 vaccination and was the offspring of patient 1 and patient 2. His symptoms were relatively mild when compared to those of patient 1 and patient 2, and his initial C-reactive protein (CPR) level was 5 mg/L (reference value <8 mg/L)

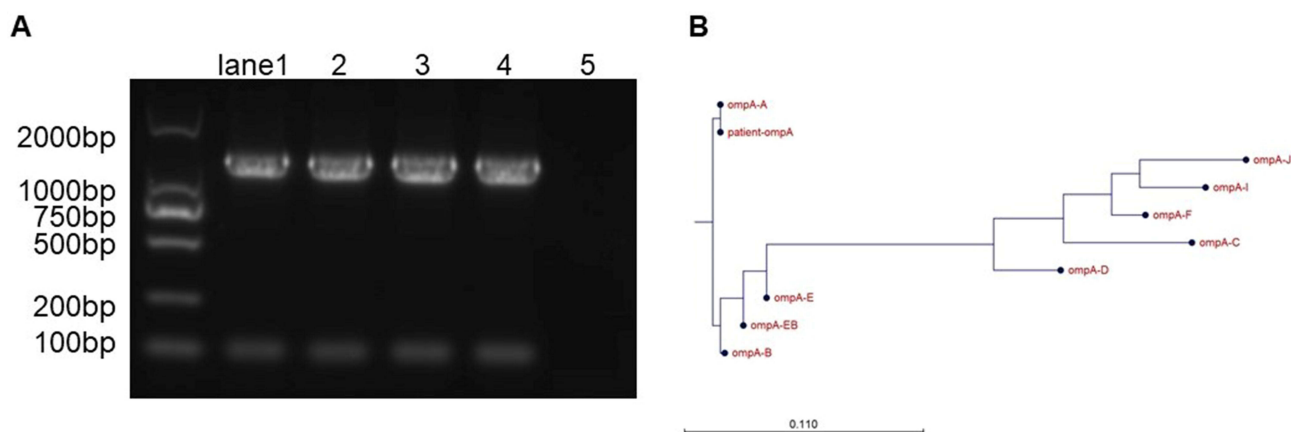


Figure 2 (A) Gel map of amplification products. Lanes 1, 2, 3, and 4 are PCR products with nucleic acid template amounts of 0.25µL, 0.5µL, 1µL, and 1.5µL, respectively, and lane 5 is a negative control. (B) Alignment of *ompA* gene from patient 1 with all *ompA* types.

(Table 1). However, his chest CT scan revealed a solid shadow in the lower lobe of the right lung which was actually more extensive than that of the parents (Figure 1F). He was prescribed azithromycin (240 mg twice a day) based on his parents' test results.

Discussion

C. psittaci is a gram-negative, intracellular parasitic pathogen that primarily infects birds but shows occasional transmission to human.² Notably, there were fewer reports on the clustered onset of *C. psittaci* in China,¹¹ which might be associated with a lower prevalence of pathogen carriers among Asian parrots compared to European parrots¹⁵ This study reports a case of a family outbreak of atypical pneumonia caused by *C. psittaci* during the COVID-19 pandemic.

In this study, it took only three days for the diagnoses to be confirmed after the onset of symptoms. Since late 2019, COVID-19 has emerged as a highly prevalent respiratory pathogen in humans, prompting patients to have heightened concern regarding respiratory symptoms such as fever, coughs, and dyspnea. In this context, the three patients in our study sought immediate medical attention at a nearby emergency department after experiencing a family-wide fever cluster. After inquiring about the patients' history of exposure to a parrot, the attending emergency department physician arranged for mNGS testing, enabling a swift and accurate diagnosis of the ailment.

The diagnosis of psittacosis pneumonia has traditionally relied on a history of contact with infected birds and the detection of pathogens in bronchial secretions. The primary diagnostic tests for psittacosis include serological assays, pathogenic cultures, and polymerase chain reactions (PCRs). Serologic testing is not a viable option for diagnosing psittacosis during the acute phase, and pathogenic culture is susceptible to specimen contamination and has a low positivity rate. And although PCR detection is both accurate and expeditious, its reliance on identifying a specific pathogen renders it susceptible to overlooking singular pathogens.¹⁶ In contrast, mNGS is a non-culture-based, next-generation pathogen detection technology that boasts rapid and efficient detection of clinical samples, a high rate of positive pathogen detection, swift detection speed, broad coverage, and heightened sensitivity. Therefore, mNGS has widespread application in identifying unknown pathogens, including genetic diseases, tumors, infectious diseases, and in noninvasive prenatal screening.¹⁷ If intracellular bacteria or rare pathogens are detected, they should be considered as causative agents even if the overall sequence number is low.

All three patients in the current study presented fever and weakness, two adult patients also experienced gastrointestinal symptoms, including nausea, poor appetite, and diarrhea, which was similar with previously reported cases.¹⁸ The laboratory data of patients showed normal or slightly elevated neutrophilic granulocyte percentage and PCT, along with high hsCRP levels. This might be due to the fact that *C. psittaci* is more pathogenic and reproduces faster than other chlamydiae,¹⁹ resulting in a more severe inflammatory response caused by *C. psittaci*. Chest CT scans revealed patchy, solid lesions in a single lung lobe in all three patients, consistent with prior research. Notably, one of the patients was a 7-year-old child who displayed milder symptoms than his parents but actually had a more extensive lesion on chest CT, suggesting a combination of mild clinical symptoms but more severe imaging signs in pediatric pneumonia.

Previous research has indicated that tetracycline is the preferred therapeutic option for treating *C. psittaci* infections.²⁰ Azithromycin has also demonstrated efficacy in this condition. Conversely, quinolones have limited inhibitory effects and are only suitable as a secondary treatment option.²¹ Macrolide antibiotics, including azithromycin, are deemed optimal for patients who cannot tolerate tetracyclines. In cases of severe, life-threatening infections, a combination of tetracycline, macrolides, and quinolones may be necessary. Following administration of tetracycline antibiotics, a reaction typically occurs within 24–48 hours, often manifesting as a decrease in body temperature. Treatment regimens are recommended to be at least 14 days, and preferably 21 days, to minimize the risk of recurrence. In the present case, the two adult patients were administered moxifloxacin for two days without significant reductions in body temperature, and their chest CT scans revealed increases in pulmonary consolidation. Upon confirmation of *C. psittaci* infection, the two adult patients were treated with doxycycline, and the pediatric patient was treated with azithromycin. Following these treatments, all patients' temperatures stabilized immediately. A follow-up assessment six months later demonstrated complete absorption of the pulmonary lesion on CT scans.

In our study, it was unfortunate for these three patients in this family outbreak. The implementation of social isolation and the imposition of recreational limitations as a result of the COVID-19 pandemic may result in a heightened desire for pet ownership. Concurrently, the prevalence of COVID-19 has led to a reduction in non-essential travel, thereby increasing the likelihood of familial clustering and the potential for a family-wide outbreak of psittacosis. On the contrary, fortuitously, the COVID-19 pandemic has instilled a heightened sense of health consciousness among patients, specifically regarding respiratory symptoms. In our study, the three patients promptly sought medical intervention within a mere 12-hour timeframe upon encountering a fever cluster affecting their entire family. This expeditious response allowed for extended periods of diagnosis and treatment, thereby facilitating the acquisition of an accurate diagnosis and prompt treatment within a maximum of 3 days from the onset of symptoms. Consequently, this timely intervention may potentially avert the occurrence of more severe complications.

Conclusion

This study reports a cluster of a family outbreak of atypical pneumonia caused by *C. psittaci* in China, which was very rare. The occurrence of a family outbreak during the COVID-19 pandemic may be attributed to familial aggregation resulting from the epidemic. Fortunately, the three cases reported in this study did not experience severe complications, which can be attributed to the prompt medical intervention and swift diagnosis. This finding implies the need to enhance patients' awareness and vigilance towards their health. Additionally, mNGS emerges as a valuable technique for accurately identifying pathogens causing pulmonary infections.

Ethics Approval and Informed Consent

This case report was approved by the Institutional Review Board of the Peking Union Medical College Hospital (K23C2638). Written informed consent for publication of their details was obtained from the patient and guardians.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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