# CASE SERIES Clinical Characteristics of Six Patients with Chlamydia psittaci Infection Diagnosed by Metagenomic Next-Generation Sequencing: A Case Series

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Abstract: The incidence of psittacosis infection has gradually increased in recent years. Metagenomic next-generation sequencing (mNGS) can be used to comprehensively identify the total DNA and RNA content of the microbiome, as well as identify both known and unexpected pathogens within 24 hours. We diagnosed and treated six patients with psittacosis infection using mNGS, two of whom developed severe disease and most of whom presented with pulmonary symptoms. One of the young female patients also presented with irregular vaginal bleeding and myocarditis. Patients with underlying gastric disorders first showed gastrointestinal symptoms, which is a rare manifestation in patients with psittacosis. Older patients with underlying disease usually showed more severe symptoms. However, rare complications can also occur in immunocompetent young people and develop into severe disease. All patients showed significant congestion at bronchial lumen lesions, which may be associated with a severe inflammatory response to mucosal Chlamydia psittaci (C. psittaci) infection. Overall, mNGS is a rapid and effective tool for the clinical diagnosis of psittacois caused by C. psittaci, and early diagnosis and treatment can prevent psittacosis from developing into a serious illness. **Keywords:** Chlamydia psittaci, diagnosis, evolutionary tree, metagenomic next-generation sequencing, serotype

#### Introduction

Human psittacosis infection, also known as ornithosis or Parrot Disease, is a relatively rare zoonosis caused by Chlamydia psittaci. Zoonotic transmission of C. psittaci has been documented through contact with infected excreta and secretions, as well as through inhalation.<sup>1</sup> A recent study showed that C. psittaci has the potential to evolve, leading to human-to-human transmission via various routes, in Shandong, China.<sup>2</sup> Severe psittacosis infection is associated with a wide range of clinical manifestations, including mild influenza-like illnesses and severe atypical pneumonia with symptoms ranging from fever, cough, dyspnea, and fatigue to multiple-organ failure and rapid death. However, not all patients report a history of contact with birds or animals.3 In the last few years, metagenomic next-generation sequencing (mNGS) has been used for precise detection of diseases in medicine, as it demonstrates much greater sensitivity than traditional culture methods. Genetic analysis of C. psittaci based on the gene sequence of outer membrane protein A (ompA) has provided a comprehensive understanding of the possible sources of human infection to facilitate better prevention.<sup>4</sup> Here, we describe the clinical characteristics of psittacosis pneumonia diagnosed by mNGS, as well as demonstrating mNGS as an efficient diagnostic method. The identification of psittacosis infection serotypes via molecular methods may improve the study of psittacosis in terms of phylogeny, epidemiology, pathology, clinical diagnosis, and treatment.

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#### Case I

The first case was a 77-year-old male patient who experienced right-sided chest tightness for 3 days. After becoming disorientated and experiencing blurred vision over the following days, the patient sought medical attention. On admission, the clinical examination revealed a temperature of 39.7°C. Computed tomography (CT) on admission revealed inflammatory lesions in bilateral lungs with ground-glass opacities (GGOs) and consolidation with a surrounding halo sign (Figure 1A, left). We collected the following laboratory test results when the patient was admitted (Table 1). Cefoperazone sodium/sulbactam sodium and arbidol tablets are empirically used to treat respiratory tract infections. The diagnosis of diabetes mellitus was confirmed after the patient underwent two blood glucose tests. The patient achieved good glycemic control on pre-mixed insulin (Mixtard) at a low dose of 5 IU/h. The patient quickly developed severe pneumonia and was transferred to the intensive care unit (ICU). Bronchoalveolar lavage fluid (BALF) was extracted for culture and drug sensitivity testing. Unbiased mNGS of BALF identified 745 specific sequence reads corresponding to *C. psittaci* and 200 sequence reads corresponding to *Candida glabrata* on the sixth day of hospitalization. We first administered cefoperazone sodium/sulbactam sodium and meropenem, followed by doxycycline plus azithromycin to treat psittacosis. Itraconazole was used to treat infection with *C. glabrata*, while traditional Chinese medicine Xuebijing injection was used to reduce systemic inflammation. Obvious lesion absorption in bilateral lungs was observed 25 days after onset (Figure 1A, right). The patient had started housing a Chinese starling 1 month before symptom onset.

#### Case 2

A 23-year-old female patient experienced a fever and chills after exposure to a cold environment and had a body temperature of 40°C. She exhibited several other symptoms, including dizziness, sweating, headache, chest pain, sore throat, nasal congestion, bloody sputum, general fatigue, and muscle soreness. The patient had finished her menstrual period 10 days prior, but after admission, she developed irregular vaginal bleeding that lasted for 4 days; however, there were no abnormalities on gynecological examination. CT images obtained from the patient in Case 2 observed halo signs, as well as GGOs of bronchial inflation, in the left lower lobe of the lung (Figure 1B, left). Urine antigen testing for Legionella pneumophila was negative. At the beginning of treatment, the patient was treated with arbidol tablets, budesonide, and other symptomatic treatments. The patient experienced fever, chest pain, non-specific T-wave abnormalities, and vaginal bleeding on the third day of hospitalization, and she was strongly suspected of having myocarditis. Abnormal laboratory test results were obtained, as follows: creatine kinase (CK), 287.33 U/L (normal range 24-170 U/L); lactate dehydrogenase (LDH), 264.52 U/L (normal range 100–240 U/L); and hydroxybutyrate dehydrogenase, 199 U/L (normal range 74–199 U/L). Cardiac troponin I (1.114 ng/mL) was also above the reference range (0-0.04 ng/mL). The patient was transferred to the ICU. The Weil-Felix test was significantly positive, with the following results: O antigen, 1:320; H antigen, 1:160; paratyphoid B, 1:80; paratyphoid C, 1:160; O antigen-X19, 1:80; and O antigen-X2, 1:80. The patient was then treated with cefoperazone sodium/ sulbactam sodium plus moxifloxacin. Four days after admission, the patient underwent bronchoalveolar lavage. The mNGS of BALF reported 15 sequence reads of C. psittaci (Table 1) and 3 sequence reads of Streptococcus pneumoniae. Moxifloxacin was continued for 10 days. A re-examination by lung CT 15 days after admission showed that the left lower lobe lesions had improved and absorbed (Figure 1B, right). The patient was a researcher, and she collected wild pig feces samples in the 2 weeks prior to symptom onset. Infected wild birds or the environment was the most likely source of C. psittaci.

#### Case 3

A 59-year-old female patient experienced recurrent high fever (40°C) with chills after feeding her chickens. She complained of muscular stiffness, dry throat, dizziness, and headache, and she visited the local clinic. The patient was treated with penicillin and cephalosporin for 2 days, but no improvement in symptoms was noted. The laboratory results at admission are shown in Table 1. Cardiac enzymes showed elevated CK (932.62 U/L), LDH (284.85 U/L), and myoglobin (106.83 ng/mL) (normal range 14–65.8 ng/mL) concentrations. Chest CT showed peripheral GGOs with vascular enlargement representing halo signs in the posterior segment of the right lower lobe, as well as small bilateral pleural effusion (Figure 1C, left). After admission, moxifloxacin treatment was administered for anti-infection due to the patient's history of avian exposure. Sodium chloride was injected for adequate rehydration, and potassium chloride was administrated to maintain rehydration and electrolyte balance. mNGS of

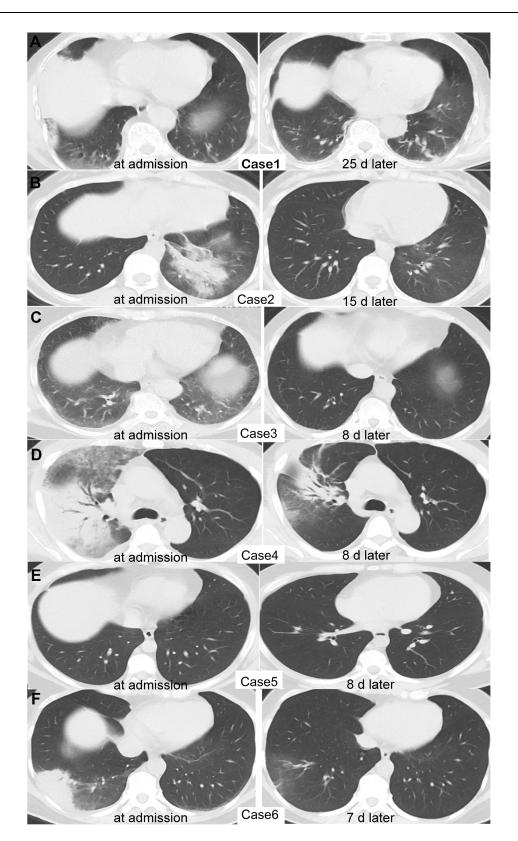


Figure I Chlamydia psittaci appearance on chest CT in Cases I-6 (A-F) on the day of admission (left) and after treatment (right).

BALF detected 803 specific *C. psittaci* sequences (Figure 2A). The patient continued treatment with a full course of doxycycline plus moxifloxacin. To trace the source of *C. psittaci*, swabs were collected from the beaks and feathers of the patient's chickens and sent for mNGS. A variety of poultry pathogens were detected, including *Avibacterium paragallinarum, Blastocystis hominis,* 

#### Table I Clinical Characteristics and Laboratory Inspections Parameters of the Six C. psittaci Pneumonia Cases

| Characteristics                        | Case I   | Case 2 | Case 3 | Case 4       | Case 5 | Case 6   | Patients, n(%) | Median Value (range) |
|--|----------|--------|--------|--------------|--------|--|----------------|----------------------|
| Demographic                            |          |        |        |              |        |  |                |                      |
| Male/female                            | м        | F      | F      | М            | М      | F  | 3/6            |                      |
| Age(y)                                 | 77       | 23     | 59     | 66           | 23     | 43   |                | 51(23–77)            |
| Sample                                 | Balf     | Balf   | Balf   | Balf         | Balf   | Balf   |                |                      |
| Underlying disease                     | Diabetes | No     | No     | Hypertension | No     | Hypothyroidism<br>\chronic atrophic<br>gastritis | 3/6(50)        |                      |
| History of exposure to avian or animal | Yes      | Yes    | Yes    | Yes          | No     | Yes  | 5/6(83)        |                      |
| Hospital stays (days)                  | 15       | 14     | 11     | 16           | 12     | 12   |                | 13(11–16)            |
| Time from admission to diagnosis(days) | 5        | 5      | 3      | 2            | 2      | 5  |                | 4(2–5)               |
| Clinical manifestations                |          |        |        |              |        |  |                |                      |
| Fever>38.5°C                           | Yes      | Yes    | Yes    | Yes          | Yes    | Yes  | 6/6(100)       |                      |
| Chills                                 | No       | Yes    | Yes    | Yes          | Yes    | Yes  | 5/6(83)        |                      |
| Cough, dyspnea, hypodynamia            | No       | Yes    | No     | Yes          | Yes    | Yes  | 4/6(66)        |                      |
| Bosom frowsty, bosom painful           | Yes      | Yes    | No     | No           | No     | Yes  | 3/3(50)        |                      |
| Laboratort test                        |          |        |        |              |        |  |                |                      |
| WBC (normal 3.5–9.5×109/L)             | 8.7      | 5      | 9.7    | 7.9          | 10.8   | 6.8  |                | 8.30(5-10.8)         |
| Neutrophils ratio(normal 40–75%)       | 90.09%   | 73.98% | 83.61% | 84.62%       | 75.65% | 76.34%   |                | 80.00%(73.98–90.09%) |
| Lymphocytes ratio(normal 20–50%)       | 5.65%    | 17.04% | 8.97%  | 7.53%        | 13.12% | 16.50%   |                | 11.05%(5.65–17.04%)  |
| EO%(normal 0.8–4%)                     | 0.07%    | 0.18%  | 1.86%  | 0.07%        | 1.07%  | 0.14%  |                | 0.16%(0.07-1.86%)    |
| CRP(normal0.01-6mg/L)                  | 128.3    | 73.6   | 109.6  | 24.5         | 129.63 | 120.81   |                | 115.21(24.5–129.63)  |
| PCT (normal <0.5 ng/ml)                | 7.79     | 0.14   | 0.08   | 0.36         | 0.23   | 0.21   |                | 0.22(0.08–7.79)      |
| IL-6(normal 5.3–7.5pg/ml)              | 350.77   | NA     | NA     | NA           | 19.74  | 2.62   |                | /                    |
| AST(<40U/I)                            | 16.2     | 12.85  | 30.53  | 137.22       | 30.43  | 53.29  |                | 30.48(12.85-137.22)  |
| ALT(<45U/I)                            | 31.95    | 25.63  | 29.47  | 69.2         | 49.34  | 62.14  |                | 40.65(25.63-69.20)   |

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|                      | 1  |  |  |  |  |   |          | 1                    |
|----------------------|--|--|--|--|--|---|----------|----------------------|
| Ldh(100–240u/l)      | 281.67   | 270.71   | 284.85                                     | 380.46                                     | 290.43   | 212.68  |          | 283.26(212.68-380.46 |
| Hbdh(74–199u/l)      | 215.16   | 203.35   | 201.47                                     | 210.52                                     | 192.15   | 121.22  |          | 202.41(121.22-215.16 |
| HCY(4–15.4 umol/L)   | 22.54  | 9.31   | 13.45                                      | 0.13                                       | 0.6  | 8.74  |          | 9.03(0.13–22.54)     |
| Ct                   |  |  |  |  |  |   |          |                      |
| Ground-glass opacity | Yes  | Yes  | Yes  | Yes  | Yes  | Yes   | 6/6(100) |                      |
| Consolidation        | Yes  | Yes  | No   | Yes  | No   | Yes   | 4/6(66)  |                      |
| Halo sign            | Yes  | Yes  | Yes  | Yes  | Yes  | Yes   | 6/6(100) |                      |
| Air bronchogram      | No   | Yes  | No   | Yes  | No   | Yes   | 3/6(50)  |                      |
| Platform             | Mgiseq-2000<br>platform  | Mgiseq-2000<br>platform  | Nextseq<br>550Dx<br>sequencing<br>platform | Nextseq<br>550Dx<br>sequencing<br>platform | Nextseq 550Dx<br>sequencing<br>platform                        | Mgiseq-2000<br>platform                                 |          |                      |
| Mngs results (reads) | Chlamydia<br>psittaci(745),<br>Candida<br>glabrata(200),<br>Candida<br>tropicalis(3),<br>Human<br>gammaher-<br>pesvirus(4) | Chlamydia<br>psittaci(15),<br>Streptococcus<br>pneumoniae(3)           | Chlamydia<br>psittaci( <b>803</b> )        | Chlamydia<br>psittaci(90)                  | Chlamydia<br>psittaci(2),<br>Porphyromonas<br>gingivalis(3634) | Chlamydia psittaci(13),<br>Moraxella<br>catarrhalis(42) |          |                      |
| Treatment            |  |  |  |  |  |   |          |                      |
| Before mngs          | Cefoperazone<br>sodium<br>sulbactam<br>+meropenem  | Abidor+<br>budesonide<br>+cefuroxime<br>+Azithromycin<br>+moxifloxacin | Minocycline<br>+moxifloxacin               | Azithromycin<br>+moxifloxacin              | Moxifloxacin   | Cefpiramide<br>+doxycycline                             |          |                      |
| After mngs           | Doxycycline<br>+Azithromycin<br>+Itraconazole  | Moxifloxacin   | Doxycycline<br>+moxifloxacin               | Doxycycline                                | Doxycycline<br>+moxifloxacin                                   | Doxycycline   |          |                      |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CT, computed tomography; EO, Eosinophil, HBDH; Hydroxybutyrate-Dehydrogenase; HCY, homocysteine; LDH, lactate dehydrogenase; PCT, procalcitonin; WBC, white blood cell.

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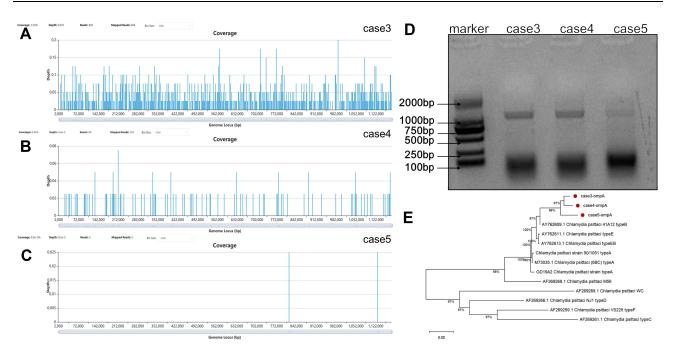


Figure 2 Sequencing results and evolutionary tree for case 3, 4, 5. (A) Genome coverage of Case: Mapping of the 864 mNGS reads from Chlamydia psittaci to reference genome. (B) Case: Mapping of the 102 reads. (C) Case 5: Mapping of the 2 reads. (D) Electrophoresis of ompA gene. PCR product sequencing showed that sequences  $\sim$ 1000 bp in size were obtained from all 3 samples. These 3 bands correspond to the expected fragment size. (E) Phylogenetic tree of ompA gene. Coverage: the percentage of the genome covered by reads over 1×. Average depth: average sequencing depth. The number of bases aligned to the reference genome divided by the length of the genome.

*Plasmodium gallinaceum, Eimeria necatrix*, and *Avian coronavirus*. However, the investigation and its results were limited because we did not swab all of the chickens. Poultry or environmental contamination was considered to be the most probable source of *C. psittaci*. After 8 days of treatment, the patient's symptoms gradually subsided. The last chest CT examination showed significant lesion absorption in both lungs (Figure 1C, right).

#### Case 4

A 66-year-old male patient experienced fever, chills, generalized aches and pains, cough, and sputum for 7 days, with a peak temperature of 39.5°C. Chest CT showed GGOs (halo sign) in the periphery of the right upper lobe and air bronchogram in the right subpleural area (Figure 1D, left). The patient had a history of hypertension. The patient was admitted with community-acquired pneumonia, which was treated with moxifloxacin and azithromycin. The patient underwent a bronchoscopic examination on the second day. The sample testing with mNGS detected *C. psittaci* (Table 1) with 90 specific sequence reads (Figure 2B). Then, we changed his treatment to doxycycline to target *C. psittaci*. The patient was treated with symptomatic and supportive treatment for 2 weeks, and the clinical symptoms of cough, sputum, and chest tightness improved significantly. CT images showed a significantly reduced lesion on the upper right lung (Figure 1D, right). The patient had visited the parrot park 2 weeks prior to symptom onset.

#### Case 5

A 23-year-old male patient experienced a dry cough for 3 days, which was aggravated at night, following a cold. He developed generalized muscle aches and fever up to 38.5°C. The laboratory results at admission are shown in Table 1. Chest CT showed right upper lobe consolidation and nodular consolidation with adjacent GGOs on admission (Figure 1E, left). The patient was treated with moxifloxacin. The mNGS results confirmed that the patient was infected with *C. psittaci* with 2 specific sequence reads (Figure 2C) and *Porphyromonas gingivalis* with 3634 specific sequence reads (Table 1). The patient showed no signs of *Porphyromonas gingivalis* infection, probably oral colonization brought in from the upper respiratory tract during sampling. Doxycycline was initiated, and the patient's body temperature returned to normal the next day. The patient was discharged

soon after in a stable condition. Chest CT showed no inflammatory exudate, and the lesions were absorbed (Figure 1E, right). The patient was unable to recall any definite living animal exposure.

#### Case 6

A 55-year-old female patient suffered from generalized body aches and pains. Fever developed, with the patient's body temperature suddenly reaching 42°C, which was accompanied by chills and rigor. The patient had black, loose, watery stools. She also complained of nausea, decreased appetite, occasional palpitations, chest tightness, chest pain, paroxysmal cough, and sputum after being treated with ibuprofen (100 mg/5 mL oral suspension). The patient had a medical history of chronic atrophic gastritis and hypothyroidism. The laboratory results at admission are shown in Table 1. The patient was treated with eprazole, monopeptide, cefpiramide, and compound amino acids to inhibit gastric acid secretion, ensure mucosal protection, provide nutritional support, and enhance immunity, respectively. Chest CT images obtained on the second day of hospitalization showed an air bronchogram in the right subpleural area with GGOs and halo signs (Figure 1F, left). The patient worked as a nanny, and her employer started housing a parrot 4 weeks before the patient's admission. The patient received anti-infective treatment with doxycycline plus cefpiramide for her symptoms because an atypical pathogen infection was considered. Bronchoscopy was performed 4 days after admission. BALF was collected and sent for mNGS, which revealed the detection of sequence reads mapped to *C. psittaci* (Table 1). After 6 days of doxycycline treatment, the patient's right upper lobe lesions improved and were totally absorbed (Figure 1F, right).

Of the patients reported, three were female and three were male, with an age range of 23–77 years (median age, 51 years). The underlying diseases differed between patients, but included diabetes mellitus (Case 1), hypertension (Case 4), and hypothyroidism and chronic atrophic gastritis (Case 6). Five of six patients had a history of recent avian or pig exposure. The patients' detailed information is summarized in Table 1. In addition, we detected *C. psittaci ompA* DNA fragments in the remaining samples from patients 3, 4, and 5, which confirmed the positive mNGS result. All three samples were greater than 1000 bp in size (Figure 2D). The polymerase chain reaction (PCR) band of Case 5 was weak, which indicated a very low *C. psittaci ompA* DNA fragment concentration in the sample. On the basis of *ompA* sequences, a phylogenetic analysis of *C. psittaci* was performed. All *C. psittaci*-positive samples shared the highest *ompA* similarity with *C. psittaci* strain 41A12, which belongs to the B genotype(Figure 2E). Detailed methods for all sample collection (Sample and Information Collection), mNGS(mNGS assay), PCR and phylogenetic tree construction

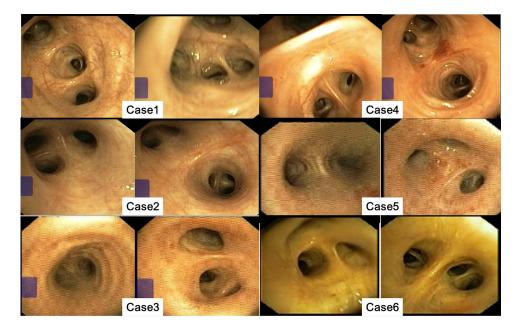


Figure 3 Bronchoscope imagings of all cases after hospitalization. All patients showed hyperemia and swelling of bronchial mucosa, significant exudation in the bronchi, and a little of white secretions in the segmental bronchi.

(PCR and Evolutionary tree construction) are given in the <u>Supplementary</u>. Significant congestion in the bronchial lumen of the lung was observed in all patients in fiberoptic bronchoscopy (Figure 3). This feature made psittacosis clearly distinguishable from *L. pneumophila* infection (Legionnaire's disease), despite their similar symptoms.

#### Discussion

Atypical pneumonia in humans can be caused by *Legionella, Mycoplasma, Coxiella*, and influenza.<sup>5</sup> When atypical pneumonia presents due to a history of exposure to birds, the diagnosis of psittacosis should definitely be considered. In our study, 83% of patients (5/6) had a history of exposure to live animal. All patients had a high fever (temperature of >38.5°C) and presented with flu-like symptoms, such as muscle aches and weakness. All patients had a rapid onset of illness. Initial blood investigations showed a weakly elevated white blood cell count of  $8.3 \times 10^9$ /L (normal range  $3.5-9.5 \times 10^9$ /L). Eighty-three percent of patients (5/6) showed an increased neutrophil percentage above the normal range. All patients had a low lymphocyte ratio. C-reactive protein was elevated in all patients with a mean value of 115.21 mg/L (normal range 0.01-6 mg/L) (Table 1). Aspartate aminotransferase and alanine transaminase concentrations were relatively normal at the time of admission and increased with psittacosis progression, but they returned to normal when the patient was discharged. Patients with psittacosis with liver involvement often present with marked hepatosplenomegaly, markedly elevated transaminases, and in some cases, intestinal discomfort. In severe cases, patients may also experience black stools. The patient in Case 6 had chronic atrophic gastritis and underwent gastric polypectomy 6 months prior to psittacosis onset. After infection with *C. psittaci*, this patient's symptoms first manifested in the digestive system.

C. psittaci infection is on the rise as animal migration and the housing of birds as pets increase. Test results identified C. psittaci as the most prevalent chlamydial species in community-acquired pneumonia in Germany.<sup>6</sup> People infected with C. psittaci experience symptoms such as fever, chills, headache, malaise, myalgia, and non-productive coughing.<sup>7,8</sup> The clinical presentation of psittacosis can vary widely in severity, ranging from asymptomatic or respiratory involvement to acute respiratory distress syndrome and death or gastrointestinal symptoms.<sup>9</sup> The patient in Case 2 had an elevated cardiac troponin I concentration that was 10-fold greater than the normal reference range (1.107 ng/mL; normal range 0–0.04 ng/mL) on admission. The main features of psittacosis pneumonia on chest radiography were Inflammatory infiltrated in the upper lung field, and GGOs were present in the bilateral lower lung fields. Nodular or consolidated shadows were commonly found in pathological areas. Lesions present on chest X-ray or CT depended on disease severity, but were not specific. All six patients had similar imaging features to those shown in previous studies.<sup>8,10,11</sup> In an animal study, in type 1 alveolar epithelial cells of the lung, C. psittaci replicated, which was followed by an influx of neutrophils, vascular leakage, and fibrinous exudation, after which the area of consolidation increased.<sup>12</sup> Air bronchograms were observed in bilateral lungs. With psittacosis progression, multiple lesions are common. The lesions can be patchy, nodular, or lumpy. They commonly have an uneven density and GGOs, interlobular thickening, and consolidation. The clinical presentation of atypical pathogenic pneumonia is similar.<sup>9</sup> In the present study, the patient in Case 2 was suspected of having community pneumonia, either caused by the Legionella genus or the Salmonella genus, before mNGS. However, pathogens were not detected in the blood or sputum or in BALF cultures, but mNGS eventually detected C. psittaci in BALF. The patient in Case 2 also had irregular vaginal bleeding, which although not heavy, has not been reported in previous studies. The gynecological consultation did not exclude that the patient's mood swings led to hormonal fluctuations causing withdrawal bleeding. C. psittaci can infect mucous membrane and invade epithelial columnar cells.<sup>3</sup> In addition to the obvious congestion of the bronchial tract, vaginal bleeding may have occurred due to vaginal mucosal involvement caused by C. psittaci.

Methods for the laboratory identification of *C. psittaci* infection include culture, serological assay, and PCR.<sup>13–15</sup> However, at most hospitals, *C. psittaci* detection methods, such as PCR, complement fixation, and serological and micro-immunofluorescence tests are not routinely available. In patients with CAP, empiric treatment does not always cover atypical pathogens. Early diagnosis and immediate treatment initiation are essential for an effective *C. psittaci* control program. As a high-throughput sequencing technology, mNGS allows for hypothesis-free and culture-independent pathogen detection directly from samples. The technique can complement current conventional identification methods in the diagnosis of *C. psittaci*.<sup>16</sup> With the widespread use of mNGS in the clinic, its use to diagnose *C. psittaci* pneumonia has increased.<sup>8,10,11,17–20</sup> All patients in the present study were diagnosed with *C. psittaci* 

infection solely using mNGS. Among the samples that were positive for *C. psittaci* detected using mNGS, three *ompA* fragments (1.4 kb) were successfully amplified and sequenced. Sample from the patient in Case 5 had minimal sequencing reads (only 2 mapping reads to the *C. psittaci* genome) and showed an ambiguous, faint DNA band. The specific reads of microbial sequences that mapped to *C. psittaci* reference genomes varied among patients, but the sequence number detected with mNGS was affected by many factors, including human background reads, sample collection, pathogen load, host depletion methods, and PCR-free library construction.<sup>21</sup> *OmpA* is commonly used for *C. psittaci* strain detection and identification.<sup>22</sup> The *ompA* sequence of *C. psittaci* obtained in this study was identical to a strain isolated from pigeon (41A12), which was classified as genotype B. Pigeons most frequently carry genotype B, but some parrots are also susceptible to this genotype.<sup>23</sup> The patient in Case 3 raised chickens, but mNGS of random samples obtained from the beaks and feathers of these chickens showed that the samples were negative for *C. psittaci*; thus, the source of infection could not be traced. The patient in Case 4 spent time in the parrot park prior to psittacosis onset and possibly contracted the infection from sick birds or from the environment. Although *C. psittaci* is an intracellular bacterium, it could survive in vitro long enough to enable transmission through the environment.<sup>24</sup>

### Conclusion

In conclusion, the epidemiology, clinical characteristics, results of mNGS, and imaging features of patients with *C. psittaci* pneumonia have been summarized, which help us to better understand and fight the disease. MNGS is a comprehensive, rapid, and newly emerging tool that assists in diagnosis of *C. psittaci*, and minimize the emergence of antibiotic-resistant. MNGS will be critical to improve the disease outcomes and prevent the potential spread of *C. psittaci*.

# **Associated Data**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

#### **Data Sharing Statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <u>https://www.ncbi.nlm.nih.gov/sra</u>, accession number.

#### **Ethics Approval and Informed Consent**

The Ethics Committees of the Second People's Hospital of Kunming approved this study. Informed consent was obtained from the patient and guardians.

## **Consent for Publication**

All six patients provided written informed consent for their case details and any accompanying images to be published.

## **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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The authors report no conflicts of interest in this work.

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