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CASE REPORT

Clinical characteristics of 14 cases of *Chlamydia psittaci* pneumonia

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SUMMARY

Among 14 patients with *C. psittaci* pneumonia, there were 9 critical and 5 non-critical cases. Ten patients improved clinically and were discharged to home; however, four patients died. Seven patients had a history of contact with birds or poultry. All 14 patients had a high fever as the presenting symptom, but most had a normal white blood cell count. Most of the patients had a significant increase in high-sensitivity C-reactive protein and procalcitonin levels. The lymphocyte count in the critical group was considerably lower than in the non-critical group. Patients in the critical group were more advanced in age than in the non-critical group. In addition, serum urea nitrogen, creatinine, procalcitonin, and lactate dehydrogenase levels were significantly higher in the critical group than in the non-critical group ($P<0.05$). The 4 patients who died had significantly increased procalcitonin levels compared to the 10 patients who survived ($P<0.05$). In summary, a high fever is usually the presenting complaint of patients with *C. psittaci* pneumonia. Such patients often progress to severe disease; however, early diagnostic confirmation by mNGS and appropriate treatment dramatically improve the prognosis. Age, lymphocyte count, procalcitonin, blood urea nitrogen, creatinine, and lactate dehydrogenase levels were shown to predict disease severity.

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INTRODUCTION

Chlamydia psittaci is gram-a negative, obligate intracellular bacterium. *C. psittaci* pneumonia, resulting from a *C. psittaci* infection, is a zoonosis that can spread among humans (Liu *et al.*, 2022). One multi-center study showed that *C. psittaci* accounted for approximately 8% of all pathogens causing community-acquired pneumonia (Wu *et al.*, 2020). Patients with *C. psittaci* pneumonia lack specific clinical symptoms, and some symptoms may be similar to those in patients with respiratory diseases caused by viral infections. In addition, standard tests do not involve the screening of *C. psittaci*. For these reasons, the misdiagnosis rate of *C. psittaci* pneumonia exceeds 50% (Liu *et al.*, 2022). The use of advanced methods such as real-time polymerase chain reaction (PCR) and metagenomic next-generation sequencing (mNGS) has significantly enhanced the detection rate of *C. psittaci* pneumonia (McGovern *et al.*, 2021; Li *et al.*, 2022a). However, the prevalence of *C. psittaci* pneumonia may be significantly underestimated worldwide due to limited reporting requirements in several countries. In health care settings, the lack of rigorous segregation protocols for the majority of patients diagnosed with *C. psittaci* pneumonia is due mainly to a high misdiagnosis rate and limited awareness of the risk of human-to-human transmission. This situation contributes to the rapid spread of *C. psittaci* pneumonia among human populations (Liu *et al.*, 2022; Wu *et al.*, 2020; McGovern *et al.*, 2021; Li *et al.*, 2022a).

We retrospectively analyzed the medical records of 14 patients with *C. psittaci* pneumonia confirmed by mNGS of serum or respiratory tract specimens. The clinical characteristics of these patients were discussed, with the purpose of raising clinician awareness of *C. psittaci* pneumonia.

MATERIALS AND METHODS

Subjects

This study was approved by the medical ethics review board of Tongling People's Hospital in May 2022 (No. 2022008). Written informed consent was obtained from each patient. We retrospectively collected the medical records of 14 patients with confirmed *C. psittaci* pneumonia who were treated in the Depart-

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ment of Respiratory and Critical Care Medicine and the Intensive Care Unit of Tongling People's Hospital from October 2019 to May 2022. The clinical data of these patients were retrospectively reviewed. *C. psittaci* pneumonia was diagnosed when the following two criteria were met:

1) adherence to the diagnostic criteria for community-acquired pneumonia;
2) detection of nucleic acid sequences of *C. psittaci* by mNGS of the bronchoalveolar lavage fluid, sputum, or blood specimens.

The diagnostic criteria for community-acquired pneumonia include the following elements:

1) community onset;
2) a recent history of cough and expectoration or a worsening of pre-existing respiratory symptoms accompanied by purulent sputum, chest pain, or dyspnea;
   a) fever;
   b) signs of lung consolidation and/or moist rales auscultated;
   c) peripheral blood leukocyte count exceeding 10×10^9/L or falling below 4×10^9/L with or without left shift;
3) chest computed tomography (CT) indication of new patchy infiltration shadows, lobular or segmental consolidation, and ground-glass opacities or interstitial changes with or without a pleural effusion. *C. psittaci* pneumonia is diagnosed when the first and third criteria, along with any of the sub-criteria of the second criterion, were met. However, certain conditions were excluded from this diagnosis, namely: tuberculosis, lung cancer, non-infectious pulmonary interstitial disease, pulmonary edema, atelectasis, pulmonary embolism, pulmonary eosinophilia, and pulmonary vasculitis.

The exclusion criteria were as follows: <14 years of age (patients under 14 years of age were admitted to the pediatric inpatient department of our hospital), patient with tuberculosis, lung cancer, interstitial lung disease, positive for COVID-19 nucleic acid, and chest CT findings consistent with COVID-19.

Data collection

Using a retrospective approach, we conducted a search within the electronic medical records to collect data relevant to epidemiology, general information, symptoms, signs, laboratory test results, radiographic findings, mNGS results, treatment, and clinical outcomes. The laboratory test indicators included the following: white blood cell count, hemoglobin concentration, platelet count, lymphocyte count (LYM), high-sensitivity C-reactive protein (hCRP), D-dimer, blood potassium, blood sodium, procalcitonin (PCT), blood urea nitrogen (BUN), serum creatinine (SCr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), and lactate dehydrogenase (LDH) levels. Any missing data in the medical records were obtained through telephone follow-up. Patients who could not be reached by telephone were excluded from the study.

mNGS

Specimens (sputum, bronchoalveolar lavage fluid, and blood) were collected as described above (Miao *et al*., 2018). Selected samples were further delivered to BGI Genomics Co., Ltd (Shenzhen, China) for mNGS.

Sample processing and DNA extraction from blood samples: 3 ml of blood centrifuge 2910 g for 10 minutes within 8 h. Next, 300 µl of plasma was used to extract the nucleic acids with TIANMicrobe magnetic bead method pathogenic microbial DNA/ extraction kit (NG550-01).

Sample processing and DNA extraction from respiratory tract samples: from a total of 1.5-3 ml of sputum/alveolar lavage fluid, 450 µL was used from each of these samples and mixed with 11.5 ml 1.0% saponin for a final concentration of 0.025% for 15 s, left at room temperature for 5 min, and then added with 75 µL to remove host reaction MIX. The reaction was further incubated at 37°C for 10 min, centrifuged 18,000 x g centrifuge for 5min, and 450 ml of supernatant removed. The remaining pellet (about 70-80Ml) was further diluted with 800 µL PBS centrifuged 18,000 x g for 5 min. The supernatant (~800 ml) was further discarded and the pellet suspended in 370 µL TE-buffer and mix-vortex. A volume of 7.2 µL Lyticase (RT410-TA, TIANGEN BIOTECH, Beijing, China) was added to each of these sample tubes along with 250 µL 0.5 mm glass beads for physical homogenization. A total of 300 µL of each sample product was further used to extract DNA with TIANMicrobe magnetic bead pathogenic microorganism DNA/RNA extraction kit (NG550-01), according to the manufacturer's instructions.

The extracted nucleic acids were used to construct DNA libraries. This includes the use of DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE50) to generate the DNA libraries via processes involving DNA fragmentation, end repair, adapter ligation, and PCR amplification. Agilent 2100 Bioanalyzer was used to check the fragment size of the libraries, which was about 300bp. Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc.) was used to measure the concentration of libraries. The libraries were pooled by equal quality based on concentration and cycled to form unit chain ring structures. DNB nanospheres were then generated by rolling cycle replication. The prepared DNB nanospheres were loaded into the sequencing chip and sequenced with BGISEQ-50/MGISEQ-2000 (Jeon *et al*., 2014).

Data analysis: low-quality reads were removed to obtain high-quality data (Base Call Quality score <20,

and short fragments <35 bp). BWA (BWA: http://bio-bwa.sourceforge.net/) was used to map clean reads to human reference genome (Li and Durbin, 2009). After removing low-complexity reads and reads that were mapped to human genome, the remaining data were compared with BGI Pathogen Metagenomic Database (PMDB) to identify the bacteria taxonomy from each sample.

Statistical analysis

The data were subjected to descriptive statistical analysis using SPSS 23.0 software. Measurement data satisfying a normal distribution are expressed as the mean±standard deviation ($x \pm s$). Continuous variables not normally distributed were described as medians (interquartile range [M {Q_L, Q_U}]) A t-test was used for intergroup comparisons of measurement data obeying a normal distribution, otherwise the Mann-Whitney U test was used. A p-value < 0.05 indicated a significant difference.

RESULTS

3.1 General information and clinical manifestations

There were 14 patients (10 males and 4 females; mean age, 62.2±11.4 years; age range, 43-81 years) with community-acquired pneumonia in which *C. psittaci* was detected. All of the cases were sporadic cases outside the hospital. These patients reported to have no contact history within 14 days with individuals suspected or diagnosed with *C. psittaci* pneumonia before onset of illness. There were 9 critical cases, 5 non-critical cases, and 4 deaths. All of the patients had an acute onset and seven had contact history with birds or poultry (4 patients had contact with parrots and 3 patients had contact with poultry). Four patients had a history of hyperglycemia, three patients had a history of hypertension, and one patient had a history of varicosities; six patients had no history of an underlying disease. All 14 patients had fevers, with a mean temperature ≥39.0°C. Five patients had a peak temperature > 40*C associated with a persistent fever. Six patients had a cough, 7 patients had dyspnea, 2 patients had a disturbance of consciousness, 2 patients had blood-stained sputum, 3 patients had fatigue, 1 patient had myalgias, and 2 patients had diarrhea. Moist rales were auscultated during physical examination of the chest in all patients.

3.2 Laboratory test findings

Five patients had an increased white blood cell count. All 14 patients had an increased hCRP level. The majority of them had a hCRP ≥200 mg/L (8/12 patients), and an increased PCT level (12 patients). Six patients had thrombocytopenia and 12 patients had significant lymphocytopenia. Five patients had an increase in the BUN level, and six patients had an increase in the SCr level, including one patient with a significant increase. Eleven patients had an increase in the creatine kinase level. All of the 14 patients had an increase in the LDH level, with 8 patients having an increase in liver enzymes (alanine transaminase and aspartate aminotransferase). The serum D-dimer level was elevated in 12 patients (*Table 1*).

3.3 Comparison of laboratory test results between the critical and non-critical groups

The lymphocyte count in the critical group was considerably lower than in the non-critical group. The disease severity was found to be more prevalent in older individuals. In addition, the BUN, SCr, PCT, and LDH levels were significantly higher in the criti-

Table 1 - *Laboratory test results of 14 cases of Chlamydia psittaci pneumonia.*

Laboratory test	*Cases*	*M (Q L, Q U) or x±s*	*Normal range*
White blood cell count ↑	5/14	9.38±3.84	(3.5~9.5) ×109 /L
Lymphocyte count ↓	12/14	0.54(0.29, 0.79)	(1.1~3.2) ×109 /L
Hemoglobin ↓	10/14	118.57±15.66	(130~175) g/L
Platelet count ↓	6/14	136.93±52.39	(125~350) ×109 /L
Procalcitonin ↑	12/14	3.07 (0.42, 10.92)	(0~0.25) ug/L
D-dimer ↑	12/13	3.81 (1.87, 6.77)	(0~0.5) mg /L
AST ↑	13/14	114 (57.75,183.5)	(9~50) U/L
ALT ↑	10/14	58.5 (35.25, 74.25)	(15~40) U/L
CK ↑	11/14	785 (318.5, 1872.0)	(50~310) U/L
LDH ↑	14/14	645 (482, 1100)	(120~250) U/L
Urea nitrogen ↑	5/14	7.25 (5.45, 10.27)	(3.6~9.5) mmol/L
Creatinine ↑	6/14	99.4 880.5, 130.43)	(57~111) μmol/L
Serum potassium ↓	11/14	3.22±0.29	(3.5~5.3) mmol/L
Serum sodium ↓	12/14	131.8±5.79	(137~147) mmol/L

Note: PCT, procalcitonin; ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase.

cal than in the non-critical group (P<0.05). The white blood cell count and creatinine, creatine kinase, and D-dimer levels did not differ between the critical and non-critical groups (*Table 2*).

3.4 Comparison of laboratory test results between the survivors and non-survivors

The patients who died had severe pneumonia, with PCT level in non-survivors significantly higher than in survivors (P<0.05). However, there was no significant difference between the non-survivor and survivor groups when considering age, the blood cell count, lymphocyte counts, and levels of LDH, BUN, SCr, and D-dimer (see *Table 3*).

3.5 Radiographic findings

All the patients had patchy or flaky hyperdensities and consolidation on chest radiographs. Ten patients had bilateral lung lesions and four patients had unilateral lung lesions. Two patients had mild pleural effusions with partially-aerated bronchus signs and partial ground-glass shadows (*Figure 1A*). The lesions demonstrated on chest CT progressed rapidly as the patients' condition deteriorated (*Figure 1B*). The inflammatory lesions were absorbed without fibrotic changes after achieving a clinical cure (*Figures. 1C, 1D, 1E, 1F*).

3.6 mNGS

All 14 patients were positive for *C. psittaci* based on mNGS (*Table 4*), including 6 sputum specimens, 1 peripheral blood specimen, and 7 bronchoalveolar lavage fluid specimens. The number of read sequences for each pathogen are shown in *Table 4*. According to previous data (Miao *et al*., 2018), mNGS of respiratory tract specimens has not revealed *C. psittaci* as contamination or background bacteria. Combined with clinical symptoms and chest CT, *C. psittaci* pneumonia was diagnosed in all 14 cases.

There was only one blood specimen, and the possibility of contamination was low. The patient in question was responsive to doxycycline therapy and the

Table 2 - *Comparison of laboratory test results between the critical and non-critical groups.*

Item	*Non-critical group*	*Critical group*	*t/Z*	*P*
Cases	5	9		
Age (year)	51.2±7.76	68.33±7.98	3.88	0.002*
White blood cell count ($\times_{10}^{9}$ /L, $x\pm s$)	7.53±2.33	10.41±4.24	1.39	0.190
Lymphocyte count [$\times_{10}^{9}$ /L, $M\ (Q_L, Q_U)$]	0.79 (0.59, 1.05)	0.41 (0.25, 0.55)	2.20	0.029*
D-dimer (mg/L, $M\ (Q_L, Q_U)$	2.45 (0.49,6.77)	4.04 (2.6,8.6)	1.08	0.33
AST $(U/L, M\ (Q_L, Q_U)$	58 (48.5,96.5)	136 (96.5,197)	1.71	0.083
Urea nitrogen [mmol/L, $M\ (Q_L, Q_U)$]	4.4 (4.25, 7.25)	9.7 (6.3, 13.2)	2.20	0.029*
Creatinine [μmol/L, $M\ (Q_L, Q_U)$]	82 (71.2, 94.9)	119 (99, 151.3)	2.2	0.029*
ALT [U/L, $M\ (Q_L, Q_U)$]	52 (26.5, 75.5)	65 (41, 75.5)	0.54	0.606
CK [U/L, $M\ (Q_L, Q_U)$]	402 (56, 771.5)	1282 (720.5, 2852.5)	1.93	0.06
PCT [ug/L, $M\ (Q_L, Q_U)$]	0.376 (0.184, 1.542)	8.827 (2.938, 11.94)	2.73	0.004*
LDH [U/L, $M\ (Q_L, Q_U)$]	473 (269.5, 493.5)	843 (645, 1170.5)	3.0	0.001*

Note: PCT, procalcitonin; ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase* *P<0.05.

Table 3 - *Comparison of laboratory test results between the death and survival groups.*

Item	*Survival group*	*Death group*	*t/Z*	*P*
Cases	10	4		
Age (year)	59±11.9	70.25±4.43	1.8	0.096
White blood cell count ($\times 10^9$ /L, $x\pm s$)	8.61±3.04	11.31±5.41	1.21	0.251
Lymphocyte count [$\times 10^9$ /L, (Q_L, Q_U)]	0.55(0.41, 0.79)	0.34(0.24, 1.09)	0.85	0.454
D-dimer [mg/L, $M\ (Q_L, Q_U)$]	3.11(1.34,4.49)	8.6(3.07,12.85)	1.69	0.106
AST [U/L, $M\ (Q_L, Q_U)$]	91(56.75,185)	137.5(94.75,184)	0.25	0.454
Urea nitrogen [mmol/L, $M\ (Q_L, Q_U)$]	6.75(4.38, 9.03)	132(7.28, 23.7)	1.84	0.076
Creatinine [μmol/L, $M\ (Q_L, Q_U)$]	94.9(73.6, 130.43)	106.05(98, 410.65)	0.99	0.374
ALT [U/L, $M\ (Q_L, Q_U)$]	68.5(33, 78.25)	48(38.5, 61.25)	0.92	0.374
CK [U/L, $M\ (Q_L, Q_U)$]	730.5(62, 2386.25)	1118.5(761.5, 1487.5)	0.71	0.539
PCT [ug/L, $M\ (Q_L, Q_U)$]	1.43(0.34, 3.71)	11.57(9.3, 41.31)	2.55	0.008*
LDH [U/L, $M\ (Q_L, Q_U)$]	549.5(422.25, 865.25)	965.5(657, 2553)	1.56	0.142

Note: PCT, procalcitonin; ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase* *P<0.05.

patient's temperature returned to normal. Although this patient was diagnosed with *C. psittaci* pneumonia, the number of sequences of this bacteria obtained from the blood specimen was too small (n=2). A high number of *Chlamydia psittaci* sequences was found in the respiratory tract system, with a maximum of 148,661 reads. The sequence data from cases 2 and 4 were used to investigate for potential drug resistance genes. However, no drug resistance genes were detected. Other pathogens, including *Acineto-*

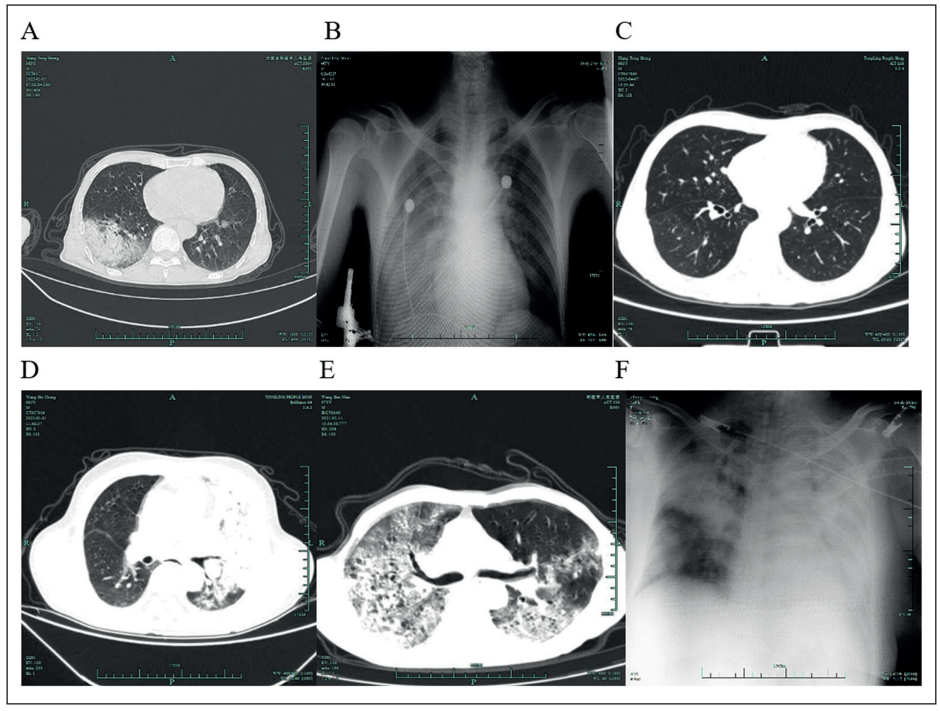

Figure 1 - *CT image of one 63-year-old patient.*
(A) shows the CT images in one 63-year-old patient at the time of admission on 7 February 2022. (B) shows the bedside chest image 5 days after admission. The lesions in the right lung progressed rapidly. (C) shows the chest CT image after the patient achieved a clinical cure; the lesion was completely absorbed. (D) indicates consolidation in the upper lobe of the left lung. (E) indicates flaky hyperdensities with ground-glass exudation in bilateral lungs. (F) indicates consolidation in the lungs bilaterally.

Table 4 - *Pathogen diagnosis by mNGS.*

Case No.	*Gender*	*Age*	*history of contact with birds or poultry*	*NGS samples*	*Pathogen (read-count)*
Case 1	Female	60	No	bronchoalveolar lavage fluid	*Chlamydia psittaci* (647) *candida parapsilosis* (448
Case 2	Male	47	No	bronchoalveolar lavage fluid	*Chlamydia psittaci* (13)
Case 3	Female	47	Contact with parrots	blood specimen	*Chlamydia psittaci* (2)
Case 4	Male	43	Contact with parrots	bronchoalveolar lavage fluid	*Chlamydia psittaci* (53)
Case 5	Male	59	Contact with the pigs after slaughter	bronchoalveolar lavage fluid	*Chlamydia psittaci* (2330)
Case 6	Male	63	History of poultry exposure in flower and bird markets	sputum specimen	*Chlamydia psittaci* (49) *Candida albicans* (282734)
Case 7	Female	59	Contact with poultry	sputum specimen	*Chlamydia psittaci* (15)
Case 8	Male	73	No	sputum specimen	*Chlamydia psittaci* (30013) *Acinetobacter baumannii* (194)
Case 9	Male	81	No	bronchoalveolar lavage fluid	*Chlamydia psittaci* (39)
Case 10	Male	57	Keep ducks	sputum specimen	*Chlamydia psittaci* (20131)
Case 11	Female	74	No	bronchoalveolar lavage fluid	*Chlamydia psittaci* (410) *Acinetobacter baumannii* (666) *Candida albicans* (29621)
Case 12	Male	67	Breeding poultry	bronchoalveolar lavage fluid	*Chlamydia psittaci* (148661) *Coccus pneumoniae* (3)
Case 13	Male	66	No	sputum specimen	*Chlamydia psittaci* (4226)
Case 14	Male	75	No	sputum specimen	*Chlamydia psittaci* (130) *Acinetobacter baumannii* (40059) *Klebsiella pneumoniae* (276)

bacter baumannii, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Candida albicans* were also detected in 6 specimens in co-infection with *Chlamydia psittaci*. Regarding the specimens of cases 11 and 14, the number of sequences (read-count) for *Chlamydia psittaci* was found relatively low or very low compared to other bacteria in co-infection (*Table 4*). For the remaining 8 specimens in which *Chlamydia psittaci* was the only pathogen found, a high number of sequences was found in 3 out of 5 (specimens from case 5, case 10, and case 13).

3.7 Treatment process and outcomes

Among the 14 patients, 5 had mild disease; the remaining 9 patients with severe disease received respiratory support. One of the patients received heated, humidified, high-flow nasal cannula oxygen therapy. All of the remaining eight patients required endotracheal intubation and mechanical ventilation. Among the severe cases, 3 received blood purification therapy, five had septic shock and were treated with vasoactive agents, and five had multiple organ failure.

Before the diagnostic confirmation, >2 empirical anti-infective medications were administered to all patients. The treatment regimen was changed to moxifloxacin (0.4 ivgtt qd) for 2 patients 2 weeks after the diagnosis was confirmed until discharge. One patient was treated with azithromycin (0.5 ivgtt qd) for 1 week until discharged from the hospital. Another patient, initially experiencing relapse after a week of azithromycin treatment, achieved a clinical cure when azithromycin was combined with doxycycline (0.1 po q12h). The remaining 10 patients were treated with a combination of doxycycline and moxifloxacin, with six improvements. All ten patients achieved a clinical cure, were discharged after re-examination by chest CT scan, and had resumed normal activities as of the last telephone follow-up.

Four patients experienced deterioration without any signs of improvement despite continuous treatment. Unfortunately, these patients were discharged from the hospital after their relatives chose to discontinue their treatment. According to telephone follow-ups, all 4 patients died within 24 h post-discharge. Among them, one patient exhibited thrombocytopenia and a coagulation disorder. Two patients initially showed improvement, but subsequently developed a secondary fungal or bacterial infection. The final patient had progressive deterioration despite aggressive treatment and then died.

DISCUSSION

C. psittaci is found mainly in parrots, pigeons, and poultry. Humans can contract *C. psittaci* by contact with *C. psittaci*-carrying birds or poultry and bacteria-infected excrement, plumes, and aerosol (de Gier *et al*., 2018). A high-risk factor for *C. psittaci* pneumonia is contact with birds or poultry (Balsamo *et al*., 2017). In the present study, seven patients had a history of contact with birds or poultry. This finding points out the relationship between *C. psittaci* infection and a history of contact with birds and poultry (de Gier *et al*., 2018; Balsamo *et al*., 2017). Therefore, *C. psittaci* infection should be considered for patients with community-onset pneumonia and a contact history with birds. Liang *et al.* (Liang *et al*., 2022) reported that 26.7% of patients with *C. psittaci* pneumonia had underlying diseases. In the present study, half of the patients had a history of hypertension or hyperglycemia, suggesting medical comorbidities as a risk factor for *C. psittaci* infection. Therefore, special attention should be given to those patients with underlying diseases.

C. psittaci pneumonia is characterized by an insidious onset and a latency stage that may last for 1-2 weeks, or even as long as 4 weeks. A previous study reported that *C. psittaci* infection may lead to a more severe systemic inflammatory response than other species of the genus *Chlamydia* (Knittler and Sachse, 2015). In our study, all 14 patients had a significant elevation of hCRP and the majority (12 patients) had an increase in PCT level. Moreover, the PCT level was significantly higher in the critical group than in the non-critical group. These results suggest hCRP and PCT as potential markers for *C. psittaci* pneumonia and the disease prognostic; further study with more samples is needed for strong statistical analysis. Also, severe pneumonia in over half of the patients, with rapid progression of the disease associated in some patients with multi organ damage, suggests a presumably high mortality rate (4/14) and the importance of early diagnosis of C. *psittaci* pneumonia. Interestingly, we observed a significant increase in PCT level in the non-survivor group compared to the survivor group, indicating that the severe inflammatory response worsened the prognosis of patients infected by *C. psittaci*. Thus, PCT level can be used to predict prognosis.

Upon infection, *C. psittaci* proliferates first in the local mononuclear phagocyte system before generating a systemic infection (Fraeyman *et al*., 2010). The clinical manifestations of *C. psittaci* infection may vary from asymptomatic for an extended period of time to respiratory failure. In some cases, there may be only fever, but no respiratory tract symptoms (Liu *et al*., 2022; Wu *et al*., 2020; McGovern *et al*., 2021; Li *et al*., 2022a; Fraeyman *et al*., 2010). Fever has been repeatedly reported as the dominant symptom of *C. psittaci* infection (Wu *et al*., 2020; McGovern *et al*., 2021; Li *et al*., 2022a; Liang *et al*., 2022). Changes in parameters for hematologic system, liver, kidney, coagulation function, and muscular system were found to be involved in all patients in the current study. Moreover, lymphocyte count, age, BUN, SCr, PCT, and LDH levels were associated with disease sever-

ity. Similar to previous studies, these results showed that drastic changes in these parameters are associated with organ failure and are important factors for the prognosis of the C. psittaci pneumonia (Liang *et al*., 2022; Chen *et al*., 2020). However, given the limited sample size, we are not able to provide strong evidence, and further studies with more enrolled patients are needed. Several studies have demonstrated that the etiologic basis of pneumonia caused by *C. psittaci* involves perivascular inflammation that extends to the periphery, resulting in lobular and interstitial pneumonia, and often with alveoli filled with cellulose or serous exudate (Li *et al*., 2022b; Shen *et al*., 2021). Among the 10 patients, bilateral patchy consolidation was observed in the lungs, and two patients exhibited pleural effusions. This manifestation is inconsistent with previous reports indicating that infection typically affects only one lung. However, this discrepancy may be attributed to the limited sample size and to the critical condition of some patients upon admission reported in this present study. As shown by previous reports, patients with *C. psittaci* pneumonia lack specific clinical symptoms and are easily misdiagnosed (Liu *et al*., 2022). Traditional microbial culture and serological detection are laborious and time consuming, and their use is currently very limited for screening C. *psittaci* pneumonia. However, mNGS has been proven to enhance the detection rate of *C. psittaci* pneumonia (Li *et al*., 2022a; Liang *et al*., 2022), providing a novel and reliable approach for microbial detection. This technique can accurately and efficiently identify suspected pathogens, thereby dramatically increasing the accuracy and timeliness of diagnosing pneumonia caused by *C. psittaci* (Wu *et al*., 2020; Li *et al*., 2022; Liang *et al*., 2022; Chen *et al*., 2020). In the current study, all patients had confirmed *C. psittaci* infections through mNGS of specimens collected from the respiratory tract or peripheral blood. Other pathogens with a potential to cause pneumonia, including *Acinetobacter baumannii* and *Streptococcus pneumoniae*, were also detected by mNGS in some patients in co-infection with *C. psittaci*. The contribution of each of these pathogens in disease severity requires further study with a sufficient sample size. facGuidelines recommend tetracycline as first-line treatment for *C. psittaci* pneumonia (Chinese Society of Respiratory Medicine, 2016). If tetracycline is unsuitable, macrolides are the second choice. Quinolones are less effective than tetracycline and macrolides (Chinese Society of Respiratory Medicine, 2016; Miyashita, 2022; Shi *et al*., 2021). Doxycyclineis a recommended tetracycline drug. However, some patients with undiagnosed psittacosis infection were treated with moxifloxacin or azithromycin, and achieved effective improvements. Upon confirming *C. psittaci* diagnosis for these patients, the same treatment was maintained. Severe *C. psittaci* pneumonia may benefit from appropriate and timely antibiotics (Chen *et al*., 2020). Without proper treatment, the mortality rate can reach 10%-20% (Rybarczyk *et al*., 2020). In this study, four patients with severe *C. psittaci* pneumonia died, suggesting that worsening prognosis that may be due to a combination with secondary infections caused by other pathogens or other complications.

The main limitation of the present study was a limited sample size. In future studies, more samples are need in order to better assess potential markers for disease prognosis and the impact of other pathogen in co-infection with *C. psittaci*. This will provide baseline data for performing strong statistical comparisons among various parameters and provide guidelines for clinicians to table this important bacterial zoonotic disease.

In summary, *C. psittaci* pneumonia exhibits diverse and nonspecific symptoms. Traditional detection methods are often insufficient. Prompt detection of this pathogen using mNGS is crucial, especially for cases with bird or poultry exposure combined with fever. Early antibiotic treatment is vital for prognosis improvement. Caution is advised to prevent secondary bacterial infections and complications during patient recovery.

Authorship statement

BC conceptualized the study and wrote the manuscript. All the authors participated in the data collection and analysis. JW, LL and DY made critical revisions to this manuscript. HW helped with the data analysis. All the authors approved the final version of this manuscript.

Declaration of conflicts of interest

The authors declare of no conflicts of interest.

Availability of data and materials

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

References

Balsamo G., Maxted A.M., Midla J.W., Murphy J.M., Wohrle R., Edling T.M., et al. (2017). Compendium of Measures to Control *Chlamydia psittaci* Infection Among Humans (Psittacosis) and Pet Birds (Avian Chlamydiosis), *J Avian Med Surg.* **31** (3), 262-282. doi: 10.1647/217-265. PMID: 28891690.

Chen X., Cao K., Wei Y., Qian Y., Liang J., Dong D., et al. (2020). Metagenomic next-generation sequencing in the diagnosis of severe pneumonias caused by *Chlamydia psittaci*. *Infection.* **48** (4), 535-542. doi: 10.1007/s15010-020-01429-0. Epub 2020 Apr 20. PMID: 32314307; PMCID: PMC7223968.

Chinese Society of Respiratory Medicine. Guidelines for Diagnosis and Treatment of Community-Acquired Pneumonia in Chinese Adults (2016 edition). Chinese Journal of Tuberculosis and Respiratory Diseases. 2016; **39** (4), 253-279.

de Gier B, Hogerwerf L, Dijkstra F, van der Hoek W. (2018). Disease burden of psittacosis in the Netherlands. *Epidemiol Infect.* **146** (3), 303-305. doi: 10.1017/S0950268817003065. Epub 2018 Jan 24. PMID: 29361998; PMCID: PMC9134569.

Fraeyman A., Boel A., Van Vaerenbergh K., De Beenhouwer H.

(2010). Atypical pneumonia due to *Chlamydophila psittaci*: 3 case reports and review of literature. Acta Clin Belg. **65** (3), 192-196. doi: 10.1179/acb.2010.040. PMID: 20669788.

Jeon Y.J., Zhou Y., Li Y., Guo Q., Chen J., Quan S., et al. (2014). The feasibility study of non-invasive fetal trisomy 18 and 21 detection with semiconductor sequencing platform. PLoS One. **9** (10), e110240. doi: 10.1371/journal.pone.0110240. PMID: 25329639; PMCID: PMC4203771.

Knittler M.R., Sachse K. (2015). *Chlamydia psittaci*: update on an underestimated zoonotic agent. *Pathog Dis.* **73** (1): 1-15. doi: 10.1093/femspd/ftu007. Epub 2014 Dec 4. PMID: 25853998.

Li H., Durbin R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. **25** (14), 1754-60. doi: 10.1093/bioinformatics/btp324. Epub 2009 May 18. PMID: 19451168; PMCID: PMC2705234.

Li H., Hao B., Wang Y., Yu D., Chen Z., Du D., et al. (2022). Metagenomic next-generation sequencing for the diagnosis of Chlamydia psittaci pneumonia. *Clin Respir J.* **16** (7), 513-521. doi: 10.1111/crj.13519. Epub 2022 Jun 20. PMID: 35724965; PMCID: PMC9329019. (a)

Li X., Xiao T., Hu P., Yan K., Wu J., Tu X., et al. (2022). Clinical, radiological and pathological characteristics of moderate to fulminant psittacosis pneumonia. *PLoS One.* **17** (7), e0270896. doi: 10.1371/journal.pone.0270896. PMID: 35816485; PMCID: PMC9273088. (b)

Liang Y., Dong T., Li M., Zhang P., Wei X., Chen H., Wang Y., Gao X. (2022). Clinical diagnosis and etiology of patients with Chlamydia psittaci pneumonia based on metagenomic next-generation sequencing. *Front Cell Infect Microbiol.* **12**, 1006117. doi: 10.3389/fcimb.2022.1006117. PMID: 36310873; PMCID: PMC9606567.

Liu S., Cui Z., Carr M.J., Meng L., Shi W., Zhang Z. (2022). *Chlamydia psittaci* should be a notifiable infectious disease everywhere. *Lancet Microbe.* **10**, S2666-5247(22)00306-8. doi: 10.1016/S2666-5247(22)00306-8. Epub ahead of print. PMID: 36372075.

Long Y., Zhang Y., Gong Y., Sun R., Su L., Lin X., et al. (2016). Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients. *Arch Med Res.* **47** (5), 365-371. doi: 10.1016/j.arcmed.2016.08.004. PMID: 27751370.

McGovern O.L., Kobayashi M., Shaw K.A., Szablewski C., Gabel J., Holsinger C., et al. (2021). Use of Real-Time PCR for *Chlamydia psittaci* Detection in Human Specimens During an Outbreak of Psittacosis - Georgia and Virginia, 2018. MMWR Morb Mortal Wkly Rep. **70** (14), 505-509. doi: 10.15585/mmwr.mm7014a1. PMID: 33830980; PMCID: PMC8030988.

Miao Q., Ma Y., Wang Q., Pan J., Zhang Y., Jin W., et al. (2018). Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice. *Clin Infect Dis.* **67** (Suppl. 2), S231-S240. doi: 10.1093/cid/ciy693. PMID: 30423048.

Miyashita N. (2022). Atypical pneumonia: Pathophysiology, diagnosis, and treatment. *Respir Investig.* **60** (1), 56-67. doi: 10.1016/j.resinv.2021.09.009. Epub 2021 Nov 5. PMID: 34750083.

Rybarczyk J., Versteele C., Lernout T., Vanrompay D. (2020). Human psittacosis: a review with emphasis on surveillance in Belgium. *Acta Clin Belg.* **75** (1), 42-48. doi: 10.1080/17843286.2019.1590889. Epub 2019 Mar 18. PMID: 30882289.

Shen L., Tian X.J., Liang R.Z., Cheng Y., Kong X.L., He F., et al. (2021). Clinical and imaging features of Chlamydia psittaci pneumonia: an analysis of 48 cases in China. Zhonghua Jie He He Hu Xi Za Zhi. **44** (10), 886-891. Chinese. doi: 10.3760/cma.j.cn112147-20210127-00082. PMID: 34565115.

Shi Y., Chen J., Shi X., Hu J., Li H., Li X., et al. (2021). A case of *Chlamydia psittaci* caused severe pneumonia and meningitis diagnosed by metagenome next-generation sequencing and clinical analysis: a case report and literature review. BMC Infect Dis. **21** (1), 621. doi: 10.1186/s12879-021-06205-5. PMID: 34193063; PMCID: PMC8243071.

Wu X., Li Y., Zhang M., Li M., Zhang R., Lu X., et al. (2020). Etiology of Severe Community-Acquired Pneumonia in Adults Based on Metagenomic Next-Generation Sequencing: A Prospective Multicenter Study. *Infect Dis Ther.* **9** (4), 1003-1015. doi: 10.1007/s40121-020-00353-y. Epub 2020 Nov 10. PMID: 33170499; PMCID: PMC7652912.