Case Report

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A severe case of pneumopathy in a duck breeder due to *Chlamydia psittaci* diagnosed by 16S rDNA sequencing

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Introduction: Psittacosis is a zoonotic infectious disease contracted from birds and caused by *Chlamydia psittaci*, an obligate intracellular pathogen. In humans, the symptoms of the disease range from inapparent illness to systemic illness with severe pneumonia.

Case presentation: A severe case of atypical pneumonia requiring extra-corporeal membrane oxygenation in a duck breeder is described. Because of the critical urgency of the case described here, and without any clear identification of the pathogen during the first days of hospitalization, treatment had to be adjusted daily. While conventional clinical methods failed to identify the causative agent, *C. psittaci* was finally identified using broad-range 16S rDNA PCR analysis performed on a sample of broncho-alveolar fluid.

Conclusion: Owing to the non-specific clinical signs of psittacosis, early identification of cases of the disease remains a challenge. *C. psittaci* should be sought in patients presenting severe acute respiratory distress syndrome without any evidence of other infectious causes and especially when exposure to birds or bird products is reported. PCR is a very useful method to help identify fastidious organisms of this kind.

Keywords: ECMO; psittacosis; severe dyspnoea.

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Introduction

Pulmonary infectious disease can lead to a severe pathological state called acute respiratory distress syndrome (ARDS) (Dreyfuss & Ricard, 2005). Treatment of this acute disease consists of lung-protective mechanical ventilation (Moloney & Griffiths, 2004) and, in the most severe cases, of extra-corporeal membrane oxygenation (ECMO) (Bonacchi *et al.*, 2012; Combes *et al.*, 2012a,b; Davies *et al.*, 2009; Lewandowski, 2000; Tiruvoipati *et al.*, 2012). Effective treatment requires regular adjustments to the anti-infectious therapy (File, 2009; Frei *et al.*, 2010; Mandell *et al.*, 2007). However, the pathogen is not always easily identifiable or even known.

Psittacosis is a zoonotic disease caused by the obligate intracellular bacterium *Chlamydia psittaci*, a bacterial pathogen characterized by a biphasic developmental cycle. Birds are the primary natural reservoir. As far as the

transmission of *C. psittaci* strains from birds to humans is concerned, members of certain professions including veterinarians, bird breeders, handlers and traders, appear to face heightened risks (Hummer *et al.*, 1988, 1992; Hinton *et al.*, 1993; Saito *et al.*, 2005; Rehn *et al.*, 2013). A typical transmission event involves inhalation of infectious dust particles during handling of infected animals, carcasses or tissues. In addition, contaminated faeces and feathers may play an essential role in zoonotic transmission.

Psittacosis causes influenza-like symptoms, associated or not with pneumopathy of variable severity (Cunha, 2006; Petrovay & Balla, 2008; Verweij *et al.*, 1995). The incubation period ranges from 5 to 30 days. Treatment with specific intracellular antibiotics such as macrolides, cyclins and quinolones is effective when administered early (Pilly, 2000; Schlossberg, 2000). Most diagnoses are established retrospectively by use of micro-immunofluorescence to test for antibodies against *C. psittaci* in paired sera. The direct detection of this microorganism is difficult as cell culturing of the patient's sputum, pleural fluid, or

Abbreviations: ARDS, acute respiratory distress syndrome; ECMO, extra-corporeal membrane oxygenation.

throat swab requires a high-containment laboratory, and no commercial nucleic acid amplification tests exist.

Here we describe a particularly severe case of pneumonia due to *C. psittaci* in a duck breeder. As initial probabilistic antibiotic therapy was not efficient and the patient's state was deteriorating, he needed to be placed on ECMO. In this study, the aetiological diagnosis was clearly established *a posteriori* using broad-range 16S rDNA PCR analysis performed on a broncho-alveolar fluid sample. Retrospective investigation conducted on the farm supported the aetiological hypothesis.

Case presentation

A previously healthy 58-year-old man was admitted to the university hospital of Angers, France on 15 December 2011, for a hypoxaemic pneumopathy. A duck farmer for 15 years, he went to Strasbourg in December 2011 for a 5 day vacation. There, he was extremely fatigued with high fever (40 °C) and became dyspnoeic with haemoptoic expectorations starting on 11 December. Once back home, he was admitted to the emergency unit on 15 December. His medical history included haemorrhoids, paracetamol allergy, and a depressive disorder with an anxiolytic treatment. At the emergency unit, the patient's respiratory rate was 30 breaths min⁻¹. A blood gas sample was taken and the results were as follows: PaO₂ 47 mmHg, PaCO₂ 31 mmHg, pH 7.51, HCO₃⁻ 24 mmol l⁻¹ in ambient air. Laboratory data revealed a white blood cell count of 7093 mm⁻³ (normal range 4000–10 000 mm⁻³), including 6639 polymorphonuclear neutrophils (normal range 1700–7500), 291 lymphocytes (normal range 1200–4000) and 163 monocytes (normal range 200–1000), platelets 157 000 mm⁻³ (normal range 150 000–400 000 mm⁻³), haemoglobin 133 g l⁻¹ (130–160 g l⁻¹), C-reactive protein 464 mg l⁻¹ (normal range <6) and procalcitonin 2.87 ng ml⁻¹ (normal range 0–0.5). The chest X-ray showed white opacity of the whole left lung. Due to the patient's serious health condition, he was transferred to the intensive care unit and an antimicrobial treatment composed of ceftriaxone (2 g IV day⁻¹) and levofloxacin (500 mg twice a day) was started immediately. He also received oxygen therapy through a facial mask (15 l min⁻¹), which improved blood gas parameters to PaO₂ 106 mmHg,

PaCO₂ 36 mmHg, pH 7.47, HCO₃⁻ 24 mmol l⁻¹. The next day, due to the persistence of severe dyspnoea, he needed high-flow oxygen therapy (Optiflow system; Fisher and Paykel Healthcare) with an O₂ flow of 30 l min⁻¹. Specific antigens of *Legionella pneumophila* gp1 and *Streptococcus pneumoniae* were not detected in urine samples collected on 16 and 18 December. Aerobic and anaerobic bacteriologic analyses of the sputum and bronchial aspirate revealed the presence of commensal flora [between 10⁶ and 10⁷ c.f.u. (ml sputum)⁻¹ and between 10⁴ and 10⁵ c.f.u. (ml bronchial aspirate)⁻¹] whereas blood samples were culture-negative. On 19 December, a CT scan showed diffuse alveolar and interstitial infiltrates with air bronchograms in the entire left lung and at the apex of the right lung (Fig. 1). During the day, respiratory function deteriorated so that the patient was intubated and placed on mechanical ventilation. A broncho-alveolar fluid sample was collected and a third antibiotic was added (erythromycin IV, 3 g day⁻¹ from 19 December). Meanwhile, as mechanical ventilation was not sufficient (despite prone or lateral positioning, inspired oxygen fraction of 100 %, 20 ppm of inhaled nitric oxide, high positive end expiratory pressure and neuromuscular blocking agents) and as the patient's health had worsened (PaO₂ 36 mmHg), a venovenous ECMO device had to be introduced on 21 December. A triple antibiotic cocktail was administered (ceftriaxone 2 g IV day⁻¹ from 15 until 26 December, replaced by imipenem 4 × 500 mg day⁻¹ until 2 January; levofloxacin 500 mg twice a day from 15 December until 9 January; erythromycin was replaced by spiramycin 3 MIU day⁻¹ from 20 December until 9 January).

The broncho-alveolar fluid sample collected on 20 December was culture-negative according to standard microbiology culture. All virological examinations returned negative findings for respiratory viruses, including H1N1 virus. Real-time PCR targeting *L. pneumophila*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were also negative. Finally, a PCR using universal primers targeting the bacterial 16S rDNA sequence (Clarridge, 2004) conducted on the DNA extracted from the broncho-alveolar fluid sample succeeded in amplifying a predicted 871 bp fragment. BLAST analysis of this sequence revealed high similarity with the 16S rDNA sequence of *C. psittaci* 6BC (GenBank accession no. CP002549.1). This result was

Fig. 1. Chest radiography (a) and computed tomography (b) of the patient's lungs.

confirmed with a *C. psittaci*-specific real-time PCR assay conducted on the clinical sample (Ménard *et al.*, 2006). Interestingly, the *ompA* sequence of *C. psittaci*-positive DNA from the patient was identical to the 06-859/1 *C. psittaci* sequence, a type strain isolated from a French duck in 2006 (E/B genotype). Analysis of a second broncho-alveolar wash, collected on 26 December after antibiotic administration, was negative using the same 16S PCR method.

Paired sera were examined for the detection of *C. psittaci*-specific antibodies using Savyon BMD-IFA (indirect immunofluorescence). An elevated titre of 128 (normal <64) for IgG and a normal titre (<16) for IgM were found on 19 December and on 24 January.

ECMO was withdrawn on 31 December. During the following days, the patient's condition stabilized despite a nosocomial super-infection with *Acinetobacter baumannii* that was successfully treated. Because of dysphagia and a non-productive cough, the patient had to be tracheostomized on 7 January. As he was suffering from peripheral neuromyopathy, physical therapy was recommended and performed during his stay in hospital. The tracheostomy was removed on 20 January and the patient was released from the intensive care unit on 26 January. Very fatigued and suffering from persistent myalgia, he returned home a month later. Upon returning to work a few weeks later, he was unable to remain on the job due to extreme fatigue. In a deeply depressive state, he had to be admitted to a psychiatric hospital, where he remained for 2 weeks. Thanks to the support of his family, he was then able to return home and his mood improved.

A retrospective veterinary investigation was conducted on the patient's farm, where two mixed duck flocks dedicated to egg production were bred. Whereas the patient had not reported any animal health problems, the veterinarian had noticed a decrease of approximately 30 % in egg production in each flock, with the presence of soft egg shells within the 2–3 weeks which preceded the onset of clinical signs in the duck farmer (Fig. 2). Next, a coughing phase was noticed in

the two flocks (from 26 to 31 December 2011 in flock 1 and from 9 to 13 January 2012 in flock 2). Several cases of necrotic enteritis and conjunctivitis in male ducks were also observed during this period. An antibiotic treatment (amoxicillin and/or doxycycline) was orally administered to the mixed flocks and the clinical signs disappeared. PCR analyses were only performed in June 2012, 6 months later, on those animals that were present at the time and still housed on the farm. Whereas cloacal swabs from ducks (30 animals per flock) and loose straw (pooled droppings, one per flock) were negative, the presence of *C. psittaci* was detected, at a low level, in dust collected in one of the two breeding areas.

Discussion

In France, about 20 severe human cases of psittacosis are reported each year by the French Reference Centre. While psittacosis is a systemic disease that often causes fever, headache and pneumonia, it rarely requires mechanical ventilation and even less often ECMO. This extra-corporeal technique provides respiratory support oxygen to patients whose lungs are so severely damaged that they cannot serve their function any longer.

Owing to non-specific clinical signs, early identification of psittacosis cases remains a challenge. Infection is generally diagnosed by evidence of sero-conversion in paired acute and convalescent phase sera. However, because of the critical urgency of the case described here, and without any clearly identified pathogen during the first days of hospitalization, treatment had to be adjusted daily. Although the occupation of the patient was known by the clinicians, the severity of the symptoms did not lead them to consider *C. psittaci* as the probable cause of infection. The best therapy would have been a cyclin, but fluoroquinolones and macrolides should also have been efficient (Jaton & Greub, 2005; Senn *et al.*, 2005; Lamoth & Greub, 2010). Due to the apparent non-beneficial impact of the two initially administered antibiotics (β -lactam and

Fig. 2. Schematic representation of events occurring in the patient and the duck flocks in November–December 2011.

quinolone) on the patient's health, the lack of identification of a causative pathogen, and since antibiotic-resistant pathogens had already been reported in farm animals and breeders (Beaujean & Vandenbroucke-Grauls, 2006; Gyles, 2008), the initial antibiotic cocktail was expanded to include macrolides and carbapenem, and the patient finally recovered.

Resistance to classical antibiotics is emerging among *Chlamydiae*. While antibiotic susceptibility data are scarce for *C. psittaci*, stable tetracycline-resistant *Chlamydia suis* strains have recently been identified in pigs (Andersen & Rogers, 1998; Di Francesco *et al.*, 2008; Borel *et al.*, 2012; Schautteet *et al.*, 2013). Unfortunately, no biological sample from the patient was available to attempt *C. psittaci* culturing. This would have been of great interest for antibiotic susceptibility determination.

Asymptomatic carriage of *C. psittaci* in ducks is widespread in French flocks. To date, two circulating genotypes have been identified (C and E/B genotypes). The detection of an E/B genotype in the broncho-alveolar fluid sampled from the patient was correlated with his occupation (Laroucau *et al.*, 2009). It should be noted that as part of his job he collected eggs daily and cleaned the premises without any protective equipment. In addition, a decrease in egg production, necrotic enteritis and conjunctivitis were reported in the ducks a few days prior to the onset of clinical signs in the patient. Most of the time, *C. psittaci* infections in ducks are asymptomatic. Co-infections with another bacteria or viruses have already been reported in turkey flocks, where they exacerbated the chlamydial shedding in infected birds (Van Loock *et al.*, 2006; Loock *et al.*, 2006). It is possible that such co-infections also occurred in the patient's flocks, leading to an increase in *C. psittaci* shedding. This may explain the clinical signs in the ducks and the subsequent severe contamination of the duck breeder. Unfortunately, no biological samples were collected from these ducks at the time of clinical onset in the patient, and the presence of *C. psittaci* was only detected, at a low level, 6 months later in dust samples collected in the farm.

In conclusion, our results show that *C. psittaci* should be sought in patients presenting severe ARDS without evidence of any other infectious causes and that PCR is a very useful method to help identify fastidious organisms. Psittacosis should be systematically considered when exposure to birds or bird products is reported. The difficulty of detecting *C. psittaci*, the delay in diagnosis, and the administration of inappropriate treatment may be important risk factors.

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