An outbreak of psittacosis due to *Chlamydophila psittaci* genotype A in a veterinary teaching hospital

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An outbreak of psittacosis in a veterinary teaching hospital was recognized in December 2004. Outbreak management was instituted to evaluate the extent of the outbreak and to determine the avian source. Real-time PCR, serologic testing and sequencing of the *ompA* gene of *Chlamydophila psittaci* were performed. Sputum samples from patients, throat-swab samples from exposed students and staff, and faecal specimens from parrots and pigeons were tested. In this outbreak, 34 % (10/29) of the tested individuals were infected. The clinical features of the infection ranged from none to sepsis with multi-organ failure requiring intensive-care-unit admission. *C. psittaci* genotype A was identified as the outbreak strain. Parrots, recently exposed to a group of cockatiels coming from outside the teaching facility, which were used in a practical class, appeared to be the source of the outbreak. One of the tested pigeons harboured an unrelated *C. psittaci* genotype B strain. The microbiological diagnosis by real-time PCR on clinical specimens allowed for rapid outbreak management; subsequent genotyping of the isolates identified the avian source. Recommendations are made to reduce the incidence and extent of future outbreaks.

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INTRODUCTION

Psittacosis is a disease caused by infection with *Chlamydophila psittaci*, an obligate intracellular bacterium. It is a zoonosis, since the main reservoir of *C. psittaci* is birds,

which can transmit the bacterium to man. Symptoms in birds range from none to overt disease. Both carriers and ill animals can shed the bacterium from many sites, including via nasal and faecal secretions. Two distinct forms of this pathogen are recognized: the infectious elementary bodies, and the fragile metabolically active reticulate bodies. Infection in man occurs when elementary bodies are inhaled. Fever, chills, headache, dyspnoea and cough usually characterize the disease in humans. The chest X-ray often shows an infiltrate (Schlossberg *et al.*, 1993; Smith *et al.*, 2005; Yung & Grayson, 1988). *C. psittaci* is classified into eight serovars (A–F, WC and M56). Serologic tests are mainly used for diagnosis, but the main drawback of the most commonly used serologic tests, such as ELISA, microimmunofluorescence (MIF) and the complement-fixation test (CFT), is that

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Abbreviations: CFT, complement fixation test; IC, internal control; MIF, microimmunofluorescence; rELISA, recombinant ELISA.

The GenBank/EMBL/DBJ accession numbers for the sequences of the *ompA* gene of the *C. psittaci* OSV outbreak strain and the unrelated *C. psittaci* CLV pigeon isolate are DQ230095 and DQ230096, respectively.

they give only a retrospective diagnosis. In this study, we describe an outbreak of psittacosis in a veterinary teaching hospital. Real-time PCR allowed for rapid outbreak management and, together with serologic testing and genotyping, allowed the extent of the outbreak to be evaluated and the avian source to be determined.

METHODS

Background. On 5 January 2005, the Department of Medical Microbiology of the Academic Medical Centre, Amsterdam, was informed of an outbreak of psittacosis in a veterinary teaching hospital. Two people had already been admitted to two different hospitals for presumed psittacosis. One of them was a veterinarian, who was admitted on 14 December 2004 and had attended a postgraduate course on 30 November 2004 provided by the veterinary teaching hospital (index case). The second person was a staff member of the veterinary hospital admitted on 5 January 2005 and not involved in the postgraduate course. A third patient was admitted on 13 January 2005, when the outbreak was already recognized. Sputa of the three patients were tested positive for *C. psittaci* with an in-house real-time PCR assay in the St Antonius Hospital. The suspected source was a flock of nine cockatiels that were used in a postgraduate teaching session on 30 November 2004. These cockatiels could no longer be traced by the time that the outbreak was recognized. The cockatiels were used only once in a postgraduate teaching session, together with nine Amazon parrots and 144 pigeons from the teaching hospital. In the past, the Amazon parrots had tested negative several times for *C. psittaci* by immunoassay (QuickVue; Quidel). The exposed parrots and pigeons were used again in a practical class for veterinary students on 21 and 23 December 2004 in the veterinary teaching hospital. Some of these parrots became overtly ill in the first week of January 2005. The extent of this outbreak was investigated by offering all students and staff the possibility of serologic testing and PCR for *C. psittaci* on sputum or a throat swab. In addition, we obtained faeces or cloacal swabs from the available birds involved in the outbreak, to establish the source.

Inclusion. The following cases, for whom PCR on a throat swab and serologic testing on two consecutive serum samples could be performed, were included: the index case, all students and staff working at the Division of Avian and Exotic Animal Medicine, where the parrots were accommodated, and all students who participated in the practical class. We obtained faecal specimens from the nine parrots and cloacal swab specimens from a subset of 23 out of 144 pigeons. These 23 pigeon samples were randomly obtained from the 144 pigeons, which were held in seven cages. From each cage, at least two pigeons were sampled.

Investigations and case definition. Serological testing was performed on two consecutive sera drawn at least 2 weeks apart (*Chlamydia* IgG/A/M rELISA; Medac Diagnostika). A psittacosis case was considered serologically proven by a threefold rise in *Chlamydia* spp.-specific IgG, a twofold or greater change in the specific IgM, or a twofold increase in the specific IgG titre, in combination with a twofold increase in the specific IgA antibody titre (Persson & Haidl, 2000; Verkooyen *et al.*, 1997, 1998). The CFT was performed with a commercially available *Chlamydia* group antigen (Virion) on all recombinant ELISA (rELISA)-positive sera. A fourfold rise in CFT titre was considered a true positive result. PCR was performed on faeces (parrots) and cloacal swabs (pigeons), or throat washes, sputum and throat swabs (humans), with a recently developed and validated real-time protocol (Heddema *et al.*, 2006a). Briefly, this real-time PCR assay targets an 82 bp fragment of the *ompA* gene of *C. psittaci* as well as an internal control (IC) plasmid. Throat-wash samples (20 ml) were only obtained from a subset of

the participating students. DNA was extracted according to the guanidinium thiocyanate/silica procedure (Boom *et al.*, 1990, 2000). All participants received a questionnaire in which information was asked for on gender, age, day of disease onset, antibiotic use and symptoms (headache, fever, muscle aches, dyspnoea and chills). A person was considered to be infected with *C. psittaci* if a positive PCR result or serologic evidence of *Chlamydia* spp. antibodies could be obtained. *C. psittaci* PCR-positive samples were genotyped by sequencing of the *ompA* gene, as previously described (Heddema *et al.*, 2006b). MEGA3 was used for editing and aligning the individual sequences and for phylogenetic analysis (Kumar *et al.*, 2004). A similarity index was calculated based on the translation of a 921 bp fragment of the *ompA* gene. Reference *ompA* genotype sequences available in the GenBank database (accession numbers AY762608–12, AF269261) were included in this analysis (Bush & Everett, 2001; Geens *et al.*, 2005).

RESULTS AND DISCUSSION

Initially, 38 exposed students and staff members participated (Fig. 1). For 29 individuals (eight male, 21 female), PCR and convalescent sera were available. The mean age was 37 years (range 19–61 years).

In total, we identified 10 cases of psittacosis (Table 1). Of the tested individuals, 34% (10/29) were therefore infected. Three individuals were admitted to three different hospitals, the index case and two staff members of the faculty. They were *C. psittaci* PCR positive in sputum. One of them presented with sepsis and multi-organ failure, and was admitted to the intensive-care unit. The other two presented with community-acquired pneumonia. In these patients, blood cultures, sputum cultures and in-house PCRs for the detection of *Legionella* spp., *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* DNA were all negative, except for one sputum culture that was rejected because of poor quality and one sputum culture that grew *Staphylococcus aureus*. This pathogen was, however, not considered to be the causative agent in that patient.

Of the remaining 26 students and staff members, three were *C. psittaci* PCR positive on a throat swab and showed seroconversion in the rELISA. They remained asymptomatic or had a brief self-limiting illness (fever, headache). None of them received antibiotic treatment. A PCR on a second throat swab, 3 weeks later, was negative for two of these three students. One student remained PCR positive when sampled 3 weeks and 2 months later. Throat-wash samples were obtained from 16 students, but none of these samples was PCR positive. One throat-wash sample was obtained from a student who was PCR positive on a throat swab sample.

Four students were PCR negative, but serologically confirmed (Table 1). One of the four students did not exactly meet the definition for a positive rELISA result, because she had a 2.7-fold (instead of threefold) increase in IgG. She did not reach a threefold increase in IgG, mainly because her first serum sample (drawn 8 days after symptom onset) was already positive for IgG. The CFT on these sera showed a fourfold increase; therefore, she was included in

Fig. 1. Flow diagram of the outbreak. ICU, intensive care unit; MOF, multi-organ failure.

the analysis. Three out of these four PCR negative but serologically confirmed cases had symptoms, and received doxycycline treatment. Inhibition of the PCR in these four cases was excluded, as the IC amplified correctly. The serologic assays used in this research are genus and not species specific, and therefore do not differentiate between antibodies directed against *C. psittaci*, *C. pneumoniae* or *Chlamydia trachomatis*. Some authors have recommended the use of MIF, which uses *C. psittaci* elementary bodies as the antigen. This test should be more specific than the rELISA and CFT which we used. However, several reports have shown that the use of MIF still results in considerable cross-reactivity between the different *Chlamydia* and *Chlamydophila* species (Bourke *et al.*, 1989; Wong *et al.*, 1994). In addition, MIF is difficult to interpret for laboratory staff who do not carry out the test on a regular

basis, and reproducibility is poor. For these reasons, we did not test the serum samples of these four cases by MIF. However, the combination of the clinical picture and obvious contact with infected birds almost excluded the other two pathogens.

The reported symptoms were muscle aches (3/3), severe headache (3/3), fever (3/3), dyspnoea (2/3) and chills (2/3). All symptoms resolved rapidly with doxycycline treatment. As the day of disease onset and the date of the practical were known, it was possible to establish the incubation periods for these three students. These were 12, 12 and 14 days, respectively. One student received doxycycline from his general practitioner for suspected psittacosis, while subsequent PCR on a throat swab and serologic testing remained negative.

Table 1. Patient characteristics

NA, Not applicable.

Gender	Age	Clinical features	PCR specimen	PCR	rELISA	CFT	Incubation period (days)	Days between first and second serum samples
M	37	Sepsis	Sputum	+	+	+	NA	14
F	37	Pneumonia	Sputum	+	+	—	14	29
M	61	Pneumonia	Sputum	+	—	—	NA	46
F	26	Fever, headache	Throat swab	—	+	+	12	21
F	27	Fever, headache	Throat swab	—	+	+	12	21
F	29	Fever, headache	Throat swab	+	+	+	11	21
M	28	None	Throat swab	—	+	+	NA	41
M	35	None	Throat swab	+	+	—	NA	21
F	25	None	Throat swab	+	+	—	NA	28
F	30	Fever, headache	Throat swab	—	+	+	14	15

Six out of nine parrots were *C. psittaci* PCR positive in faecal samples. Only one of the 23 pigeon samples was PCR positive. All nine parrots and 144 pigeons received doxycycline treatment. The *ompA* gene could be amplified and sequenced directly from the clinical specimens obtained from one of the hospital patients, three students, three parrots and the pigeon. All sequences obtained from the human respiratory samples and the parrot faeces were identical to the *C. psittaci ompA* genotype A reference strain. The sequence obtained from the pigeon was similar to the reference genotype B sequence, and thus unrelated to the outbreak. The sequences of the *ompA* gene of the outbreak strain and the unrelated pigeon isolate were submitted to GenBank and designated *C. psittaci* OSV and *C. psittaci* CLV (accession nos DQ230095 and DQ230096, respectively).

We describe a psittacosis outbreak in humans and birds in which real-time PCR was used for detection of *C. psittaci*, and sequencing of the *ompA* gene for genotyping of the isolates. Six people, six parrots and one pigeon were *C. psittaci* PCR positive. Genotyping of the isolates via the *ompA* gene identified the parrots as the source of the outbreak. The outbreak strain appeared to be a genotype A strain. The identification of an unrelated genotype B of *C. psittaci* in contact pigeons emphasizes the need for strain identification in human and animal outbreaks to gain a better understanding of the epidemiology of psittacosis in humans and birds.

In this outbreak, 34 % of the tested population was infected. Huminer *et al.* (1988) and Schlossberg *et al.* (1993) have described outbreaks in which 81 % ($n=37$) and 54 % ($n=24$), respectively, of the tested populations was infected with *C. psittaci*. In our study, the spectrum of symptoms ranged from none to sepsis with multi-organ failure requiring intensive-care unit admission. This diversity of symptoms is in agreement with that described by others (Huminer *et al.*, 1988). During this outbreak, three people were admitted to three different hospitals, three students were treated by their general practitioner for psittacosis, and another four students were infected but did not require antibiotic treatment. The hospitalized patients therefore represent only a small fraction of all infected persons. In nine of the 10 affected people, two or three tests were positive. One person had a positive PCR, a twofold increase in IgG and a negative CFT. Soon after hospital admission, this patient received treatment with doxycycline and this could have diminished the antibody response. PCR performed on sputum was very helpful for rapid diagnosis in the hospitalized patients. The central laboratory facilities of the different hospitals helped in identifying this outbreak. Subsequent investigation of the outbreak and outbreak management were therefore possible.

PCR on throat swabs in symptomatic students was of limited value in detecting *C. psittaci* infection. PCR on throat swabs is often used to diagnose pneumonia (Menendez *et al.*, 1999; Ramirez *et al.*, 1996; Schneeberger *et al.*, 2004). We confirmed the earlier observation that *C.*

psittaci can be detected for a prolonged period of time in a throat-swab sample (Huminer *et al.*, 1988). It is possible that throat swabs are not representative material for lower respiratory tract infection due to *C. psittaci*. A positive PCR on a throat swab could indicate an asymptomatic carrier, while a negative PCR result on a throat swab does not rule out psittacosis. Therefore, data that validate the use of throat swabs for diagnosing the aetiology of pneumonia are needed.

C. psittaci is classified into eight serovars (A–F, WC and M56), of which six are endemic in birds. Currently, at least nine genotypes are known. Recently, it has been stated that identification of all known genotypes and the newly discovered genotype E/B is only possible by sequencing of the *ompA* gene (Geens *et al.*, 2005). By sequencing the *ompA* gene directly from clinical samples, we bypassed the need for culture. It should be mentioned that genotyping of the *ompA* gene cannot definitely prove that this was a clonal outbreak, but the clinical data together with the *ompA* sequence analysis are highly suggestive. Certain genotypes appear to be associated with specific groups of birds (Vanrompay *et al.*, 1997). We found genotype A to be responsible for the outbreak. This genotype is most often found among psittacine birds such as parrots and cockatiels. The most prevalent *C. psittaci* genotype in human infections is currently unknown. In our study, the primary source of the outbreak turned out to be the nine parrots that were used in practical teaching sessions, but outbreak management among the contact birds was hampered by lack of records of bird identification and bird transactions. The cockatiels that had been used in a practical teaching session 4 weeks prior to the outbreak together with the parrots were untraceable at the time of the outbreak. It is very likely that these cockatiels infected the parrots and the index case, since the parrots from the teaching hospital were checked on several occasions by immunoassay, and tested negative.

In many countries, psittacosis is a notifiable disease. In Europe and the USA, measures to control *C. psittaci* infections among humans and birds have been issued (Scientific Committee on Animal Health and Animal Welfare, 2002; Smith *et al.*, 2005). The maintenance of accurate records of bird-related transactions for at least 1 year is recommended. These records should include the species of bird, bird identification, source of birds, and any identified illnesses or deaths among birds. Newly acquired birds should be tested or prophylactically treated before adding them to a group. For pet birds, it is recommended that only healthy PCR-negative birds should be sold by bird retailers.

Applying (some of) the recommendations could have prevented or limited this outbreak and made it possible to trace the cockatiels. The veterinary teaching hospital has changed its policy regarding the use of birds for teaching purposes, and will now only use birds from its in-house population. The relatively poor control of the disease in birds and the broad spectrum of clinical syndromes in

people infected with *C. psittaci* raise the question of whether this micro-organism may be a much more frequent pathogen than previously considered. For accurate diagnosis, we therefore recommend the detection of *C. psittaci* infections by PCR. Real-time PCR can specifically identify the pathogen and expedite the diagnosis of psittacosis. Sequencing of the *ompA* gene for genotyping is a helpful tool for identification of the avian source, and improves our understanding of the epidemiology of this disease in birds, humans and outbreak settings.

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