Chlamydophila psittaci genotype E/B transmission from African grey parrots to humans

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Thirty-six birds from a parrot relief and breeding centre, as well as the manager, were examined for the presence of Chlamydophila psittaci. In the relief unit, 5 of 20 African grey parrots showed depression, ruffled feathers, loss of weight and mild dyspnoea. The birds received no antibiotic treatment. Birds of the breeding unit, 14 blue and gold macaws and 2 green-winged macaws, were healthy. They received doxycycline at the start of each breeding season. The manager complained of shortness of breath but took no medication. Using a nested PCR enzyme immunoassay (EIA), Cp. psittaci was detected in the faeces of all five sick birds, as well as in a nasal and pharyngeal swab from the manager. The veterinarian and her assistant became infected while sampling the parrots, as pharyngeal and nasal swabs from both were positive by nested PCR/EIA after visiting the parrot relief and breeding centre, but they showed no clinical signs of infection. Bacteria could be isolated from three of five nested PCR/EIA-positive birds, the manager and the veterinarian, but not from the veterinary assistant. Using an ompA genotype-specific real-time PCR, Cp. psittaci genotype E/B was identified as the transmitted strain. All breeding birds tested negative for Cp. psittaci. This is believed to be the first report on Cp. psittaci genotype E/B transmission from parrots to humans. In contradiction to genotype A isolates, which are thought to be highly virulent to both birds and men, the currently described genotype E/B strain apparently caused no severe clinical symptoms in either parrots or humans.

INTRODUCTION

Chlamydophila psittaci, an obligate intracellular bacterium, has six known avian serovars, all considered transmissible to humans (Andersen, 1991, 1997; Vanrompay et al., 1993). The serovars are identified by mAbs recognizing serovar-specific epitopes on the major outer membrane protein (MOMP). These serovars correspond to six genotypes that are readily distinguished using outer membrane protein A (ompA) gene sequencing, RFLP analysis of the ompA gene (Vanrompay et al., 1997), or a recently developed genotype-specific real-time PCR (Geens et al., 2005a).

Genotype A is endemic among cockatoos, parrots, parakeets and lories (Psittaciformes), and is well known as a zoonotic agent. Genotype B is endemic among pigeons. Genotypes C and D are mostly detected in non-Psittaciformes. They are highly pathogenic to domestic poultry, and are acknowledged occupational hazards for slaughterhouse workers and generally for all people in contact with poultry, especially turkeys and ducks. Genotype E isolates were first isolated during an outbreak of pneumonitis in humans in the late 1920s and early 1930s. Subsequently, genotype E isolates have been obtained from a variety of avian hosts worldwide, including turkeys, ducks, pigeons, ostriches and rhesas. Genotype F was first obtained from an American parakeet (Andersen, 1997), and 8 years later also from a Belgian fattening turkey (Van Loock et al., 2005a).

Recently, a new genotype E/B has been described (Geens et al., 2005b), which has been isolated from Italian urban pigeons, German commercial fattening Pekin ducks and Belgian fattening turkeys. Genotype E/B reacts with both the serovar E- and B-specific mAbs, and generates the ompA RFLP pattern characteristic for genotype E. However, it differs from genotype E and B by a unique combination of a guanosine residue at positions 1006 and 1021, and a cytidine at position 1022, which results in an A instead of S at position 341 in the variable segment 4 of the MOMP. Genotype E/B can only be distinguished from genotypes E and B by ompA sequencing or by the recently developed genotype-specific real-time PCR (Geens et al., 2005a). In the present study, the genotype-specific real-time PCR was used to examine the transmission of a Cp. psittaci genotype E/B strain from African grey parrots to humans.

Abbreviations: EIA, enzyme immunoassay; MOMP, major outer membrane protein.
METHODS

Background. A parrot relief and breeding centre near Antwerp (Belgium) was visited in order to investigate the presence of *Cp. psittaci* in the birds and in the manager. The parrot relief unit housed 20 African grey parrots (*Psittacus erithacus*), received from people moving houses, or elderly people unable to nurse their birds any longer. The parrots were kept in separate cages. Additionally, seven pairs of blue and gold macaws (*Ara chloroptera*) and one pair of green-winged macaws (*Ara ararauna*) were kept for breeding. Relief and breeding birds were housed in clean, heated and separate, but adjacent, rooms. Every day, the 56-year-old manager was handling the parrots in the relief unit for ~2 h, cleaning their cages, hand-feeding and even nuzzling the birds. The man felt healthy, although he complained of continued shortness of breath. He took no medication and was a non-smoker. The breeding birds were treated with doxycycline (Soludox; Eurovet) in the drinking water during the 14 days before each reproduction period, and they were healthy. However, 5 of 20 (25 %) birds in the relief unit showed depression, ruffled feathers, loss of weight and mild dyspnoea. They received no antibiotic treatment.

Samples. All birds, as well as the manager, were sampled using dacron-tipped aluminium shafted swabs (Fiers), filled with 2 ml *Cp. psittaci* transport medium (Vanrompay et al., 1992) or DNA-stabilization buffer (Roche) for chlamydial isolation or detection of the *ompA* gene, respectively. As bird capturing was to be avoided due to stress, fresh droppings were collected from each cage floor, sampling five different spots per cage. The manager was sampled by taking both pharyngeal and nasal swabs. The veterinarian and her assistant were identified as ‘by chance’ 7 weeks later at the start of a psittacosis occupational risk study, in which they voluntarily participated. In these two persons, serology was additionally performed using an ELISA with avian recombinant MOMP as a target antigen (Verminnen et al., 2006). Humans were sampled with informed consent. All swabs were kept on ice during transport and stored at –80 °C until use.

*Cp. psittaci* identification and molecular characterization. The presence of the *Cp. psittaci ompA* gene was examined using a nested PCR/enzyme immunoassay (EIA), as described by Van Loock et al. (2005b). The nested PCR/EIA allows ELISA-based detection of the amplified gene, which becomes biotin- and fluorescein-labelled during the amplification reaction. The presence of viable *Cp. psittaci* was examined by isolation in buffalo green monkey (BGM) cells, which identifies the organisms by direct immunofluorescence staining (IMAGEN; DakoCytomation), as previously described (Vanrompay et al., 1992). *Cp. psittaci* was characterized directly from the clinical specimens using a genotype-specific real-time PCR, as described by Geens et al. (2005a). The latter is a TaqMan probe-based real-time PCR able to distinguish the *ompA* gene of all six currently described avian *Cp. psittaci* genotypes A to F, plus the recently described genotype E/B.

RESULTS AND DISCUSSION

*Cp. psittaci* genotype E/B transmission from parrots to humans

In the parrot relief unit examined in the present study, nested PCR/EIA revealed 5 of 20 (25 %) birds were positive for *Cp. psittaci*, and *Cp. psittaci* could be isolated from the faeces of 3 of these. The chlamydial *ompA* gene was detected in nasal and pharyngeal swabs from the manager. Nasal and pharyngeal swabs from the veterinarian and her assistant, examined by chance 7 weeks after visiting the parrot and relief centre, also contained *Cp. psittaci* DNA. At that time, the *Cp. psittaci* IgG (H + L) antibody titres in the veterinarian and her assistant were 1/960 and 1/240, respectively.

As in birds, the infection of the manager and the veterinarian was confirmed by isolating viable bacteria. *Cp. psittaci* isolates from the parrots, the manager and the veterinarian were all characterized as *ompA* genotype E/B. However, at that time, we could not isolate live chlamydial organisms from the veterinary assistant, nor could we type the strain directly from the clinical specimens, as the genotype-specific real-time PCR remained negative. Of the three persons involved, the veterinary assistant was the one with the least close contact to the parrots, as she was only handing over the swabs to the veterinarian. In contrast to the other two persons, she never went into the cages. This could explain why she was negative for isolation and only positive by our nested PCR/EIA, which is able to detect one organism, while our genotype-specific real-time PCR has a detection limit of 10 chlamydial organisms.

During a subsequent medical consultation, the manager was advised not to take antibiotics because he apparently showed no severe clinical symptoms. Until now, his clinical condition has not changed. However, the manager decided to close the parrot relief unit, not only due to our findings, but also for economic reasons, as heating costs became too high. All birds of the relief unit were treated with doxycycline and subsequently given to close associates of the manager who were specialist *Psittaciformes* bird breeders. So far, there have been no reports on sickness or abnormal mortality in these birds. However, as previously mentioned by Heddema et al. (2006), the maintenance of accurate records of bird-related transactions for at least 1 year should be recommended, and every new bird brought into a colony should be tested for *Cp. psittaci*. Tracking records should include the species of bird, bird identification, source of bird and any identified illness. Upon closure of the relief unit, the manager became negative for the nested PCR/EIA and for *Cp. psittaci* isolation.

As in the birds and the manager, the infection caused no severe clinical symptoms in the veterinarian and her assistant. In fact, the latter two persons were unaware of being infected. Therefore, they received no antibiotic treatment. Both persons became negative for the nested PCR/EIA and for isolation 10 weeks after visiting the parrot relief and breeding centre. However, at that time, the antibody titres in the veterinarian and her assistant were still 1/960 and 1/120, respectively.

**Psittaciformes: a higher risk to human health than other bird species?**

Birds of the order *Psittaciformes* seem to be highly susceptible to *Cp. psittaci* infection, as most veterinary
case reports are about such infections in *Cacatuidae* (cockatoos) and *Psittacidae* (parrots, parakeets, lories), dealing with severe clinical signs, such as eye discharge or swelling, laboured breathing, diarrhoea, poor appetite, lethargy, ‘fluffed up’ appearance, weakness, and even mortality (Bracewell & Bevan, 1986; Chahota et al., 2006; Kaleta & Taday, 2003). Many of the birds become chronically infected but show no clinical signs until stressed. These birds often shed *Cp. psittaci* intermittently and serve as a source of infection for humans and other birds. In particular, Amazon parrots (88%), macaws (87%), budgerigars (81%), cockatoos (80%), conures and Senegal parrots (78%), African grey parrots and eclectus (75%), grey cheek parrots (70%), lovebirds (68%) and cockatiels (65%) are highly infected, as demonstrated by serology (Fudge, 1997).

However, we do not really know if *Psittaciformes* are more susceptible to *Cp. psittaci* than are other bird species. It might be that the disease is simply noticed and therefore diagnosed more often, due to the severe clinical signs in these birds. For instance, when comparing epidemiological data of chlamydiosis in *Psittaciformes* and pigeons, the prevalence seems to be comparable, ranging from 16 to 81% and 23 to 85%, respectively (Fudge, 1997; Trávnícek et al., 2002). However, chlamydiosis in pigeons is less severe, and mortality is due more to secondary infections, such as salmonellosis and trichomoniasis.

In fact, the overall clinical picture in a given species is the result of strain virulence and host immunogenetics. We do not know much about bird immunogenetics, but we do know that *Cp. psittaci* genotypes A, B and F have been isolated from *Psittaciformes*. However, *Psittaciformes* are most frequently infected with genotype A strains, which are highly virulent, intensively excreted, and often cause mortality (Andersen, 1991; Vanrompay et al., 1997). Pigeons on the other hand, mostly become infected by genotype B and sometimes genotype E strains. However, the term genotype only refers to the sequence of the *ompA* gene, which encodes for the *Cp. psittaci* immunodominant MOMP. We do know that the MOMP is a porin and a possible bacterial adhesin, but the direct contributions of this protein to the virulence of the bacterium are not clear. We do not know why *Psittaciformes* are most frequently infected with genotype A strains, which apparently cause severe disease and mortality in these birds, and why pigeons are most frequently infected by other genotypes that are apparently less virulent. The question could probably be solved if we knew more about host immunogenetics, bacterium–host cell interactions and bacterial virulence factors such as the type three secretion system, which was recently discovered in *Cp. psittaci* (Beeckman et al., 2006).

**Impact of clinically unnoticed *Cp. psittaci* transmission to humans**

Given the fact that *Psittaciformes* are most frequently infected by genotype A strains, and the proven zoonotic transmission of genotype A strains (Heddema et al., 2006), we were surprised to find genotype E/B in both parrots and humans. So far, genotype E/B has only been discovered in pigeons, ducks and turkeys, and all these birds were clinically healthy except for the turkeys, which showed mild respiratory symptoms. In this study, psittacosis infection was actually unnoticed in all three persons involved. It may be that the E/B strain was indeed less virulent, although zoonotic transmission occurred. On the other hand, frequent exposure to *Cp. psittaci* could also explain the rather asymptomatic course of infection in these persons. However, we do not know anything about natural protection following *Cp. psittaci* infection in humans. For *Chlamydia trachomatis* infection in humans, protection against infection with the same *ompA* genotype is thought to last ~6 months. In animals, it has been demonstrated that fattening turkeys can become infected with two different genotypes during one production period of 15 weeks (Van Loock et al., 2005a). *Cp. psittaci* infection can cause severe disease in humans. However, these cases are probably only the tip of the iceberg. What lies underneath are less severe, clinically unnoticed infections, which are misdiagnosed due to symptoms similar to those of other respiratory pathogens, or even asymptomatic infections. The impact of these types of *Cp. psittaci* infections on human health is difficult to determine. We can try to extrapolate our knowledge on avian infections to human psittacosis. As in birds, carrier status might occur, as well as pathogenic interactions with other respiratory pathogens (Van Loock et al., 2006a, b).

**Frequent use of tetracyclines in *Psittaciformes***

Notwithstanding possible air contact with infected birds in the relief unit, all breeding birds were *Cp. psittaci*-negative in both nested PCR/EIA and bacteria isolation. Regular doxycycline treatment might have prevented spread of the infection to the adjacent breeding unit. With the risk of developing tetracycline-resistant strains, as described for the *Cp. psittaci*-related species *Chlamydia suis* (Dugan et al., 2004, 2007), this is surely not to be recommended as a preventive strategy. However, there is no avian *Cp. psittaci* vaccine; therefore, owners of *Psittaciformes* frequently use tetracyclines for any case of respiratory disease, or even prophylactically. One of the problems is that it is possible to buy these drugs on the internet, even without a prescription, as this is not needed in every country. However, most pet-bird owners are unaware of the dangerous situation that they might create by using these drugs frequently. They are ignorant of tetracyclines being the drugs of choice when treating human psittacosis, and the fact that the mortality rate prior to the advent of antimicrobial treatment was ~15–20%. Information campaigns on antibiotic use in pet birds with respect to zoonotic agents are urgently required, and worldwide access to antibiotics has to be restricted to veterinarians and medical doctors.
Conclusions
The number of human psittacosis cases is underestimated. For accurate diagnosis in both birds and humans, we recommend nucleic-acid-amplification tests, which specifically detect \textit{Cp. psittaci} at high sensitivity and allow molecular characterization. Parrots are often infected with genotype A strains, which are thought to be highly virulent for both birds and humans. The current report is believed to be the first on \textit{Cp. psittaci} genotype E/B transmission from parrots to humans. In contrast to reports on genotype A zoonotic transmission, the present genotype E/B strain caused no severe clinical symptoms in either birds or humans.

REFERENCES


