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Caroline van Droogenbroeck, Delphine S.A. Beeckman, Kristel Verminnen, Maja Marien, Hans Nauwynck, et al.. Simultaneous zoonotic transmission of *Chlamydophila psittaci* genotypes D, F and E/B to a veterinary scientist. *Veterinary Microbiology*, 2009, 135 (1-2), pp.78. 10.1016/j.vetmic.2008.09.047 . hal-00532498

HAL Id: hal-00532498

<https://hal.science/hal-00532498>

Submitted on 4 Nov 2010

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Accepted Manuscript

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PII: S0378-1135(08)00392-1
DOI: doi:10.1016/j.vetmic.2008.09.047
Reference: VETMIC 4177

To appear in: *VETMIC*

Please cite this article as: Van Droogenbroeck, C., Beeckman, D.S.A., Verminnen, K., Marien, M., Nauwynck, H., de Thibault de Boesinghe, L., Vanrompay, D., Simultaneous zoonotic transmission of *Chlamydophila psittaci* genotypes D, F and E/B to a veterinary scientist, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2008.09.047

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Short communication

Simultaneous zoonotic transmission of
Chlamydophila psittaci genotypes D, F and E/B to a
veterinary scientist

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Abstract

Two groups of five one-day old conventional turkeys were housed in negative pressure stables to become experimentally infected with Avian Metapneumovirus (aMPV) and *Ornithobacterium rhinotracheale* (ORT) at the age of three weeks. However, during the first two weeks, turkeys started to show respiratory disease characterized by rhinitis and dyspnoea. Routine bacterial and viral diagnoses remained negative. Therefore, pharyngeal swabs from the turkeys and from the veterinary scientist handling the animals were examined for the presence of *Chlamydophila (C.) psittaci* by using a combination of cell culture, nested PCR and *ompA* genotype-specific quantitative real-time PCR, as well as by serology. Results revealed simultaneous transmission of *C. psittaci* outer membrane protein A (*ompA*) genotypes D, F and E/B from infected turkeys to the veterinary scientist.

Keywords

Chlamydophila psittaci, respiratory disease, zoonosis, turkeys, genotyping, diagnostic methods

1. Introduction

Chlamydophila (C.) psittaci causes respiratory illness in birds and psittacosis or parrot fever in humans (Andersen and Vanrompay, 2000). All currently known avian *C. psittaci* outer membrane protein (*ompA*) genotypes are transmissible to humans. Fever chills, headache, dyspnoea and cough usually characterize psittacosis. The chest X-ray often shows an infiltrate (reviewed in Harkinezhad et al., 2008). However, the disease seems to vary considerably in severity as the clinical features of the infection can range from none to sepsis with multi-organ failure requiring intensive-care-unit

admission. People usually contract the infection by inhaling an aerosol of droppings of infected birds. In the present paper, the simultaneous transmission of *C. psittaci ompA* genotypes D, F and E/B from infected turkeys to a veterinary scientist is described.

2. Materials and methods

2.1. Case description and sampling

Conventional embryonated turkey eggs (T9 Webster) were transported from the hatchery to the Faculty of Veterinary Medicine (Ghent University), where they were incubated until hatching. Subsequently, two groups of five one-day-old turkeys were transferred to negative pressure stables to become experimentally infected with Avian Metapneumovirus (aMPV) and *Ornithobacterium rhinotracheale* (ORT) at the age of three weeks. However, during the first two weeks, turkeys started to show respiratory disease characterized by rhinitis and dyspnoea. One diseased turkey per group was euthanized in order to determine the aetiology of the infection. Necropsy revealed severe congestion of the conchae, sero-mucous rhinitis and congestion of the trachea. Routine bacterial and viral diagnoses including ORT, *Escherichia coli* and aMPV were negative. Therefore, the remaining 3-week-old turkeys were sampled (time point 1; t1) for *C. psittaci* diagnosis. Turkeys were first sampled for cell culture using one pharyngeal rayon-tipped swab (Fiers, Kuurne, Belgium) for sampling all four turkeys in each of the two groups. Swabs (n = 2) from both groups were subsequently placed in chlamydophila transport medium (sucrose 74,6 g/l (Acros Organics, Geel, Belgium); KH₂PO₄ 5,1 g/l and K₂HPO₄ 1,2 g/l (Sigma, Bornem, Belgium); L-glutamic acid mono potassium salt 0,9 g/l and gentamycin 50 µg/ml (Invitrogen, Merelbeke, Belgium); vancomycin 100 µg/ml and streptomycin 100 µg/ml (Soenen,

Merelbeke, Belgium); nystatin 25000 U/ml (Sigma) pH 7) and stored at - 80°C. Turkeys were then sampled for chlamydial DNA detection, again using one swab per group. The swabs (n = 2) were stored at - 80°C until tested. The veterinary scientist taking care of the animals, and who performed necropsies on two-week-old diseased turkeys, showed no clinical signs. Nevertheless, she was advised to seek medical attention. As for the turkeys, two pharyngeal swabs were taken from the scientist, one for cell culture and one for chlamydial DNA detection. The veterinarian additionally provided serum samples at t1 and two months later, which were examined by an antibody enzyme-linked immunosorbent assay (ELISA) using the *C. psittaci* recombinant major outer membrane protein (MOMP) as target antigen (Verminnen et al., 2006).

All animals were euthanized, placed in sealed biohazard containers and transported to an incinerator by a certified company. Two weeks later (time point 2; t2), the veterinarian still felt healthy and again pharyngeal swabs were taken to be tested for the presence of *C. psittaci*.

2.2. *C. psittaci* detection

Human (t1, n = 1) and avian swabs for culture (t1, n = 2) were inoculated onto Buffalo Green Monkey (BGM) cells (Vanrompay et al., 1992) and on post-inoculation day 3, growing chlamydial organisms were identified by indirect immunofluorescence staining using a family-specific monoclonal antibody (MAb) (7B6III) against the chlamydial major outer membrane protein, as described previously (Geens et al., 2005a). The number of infected cells was counted in five randomly selected microscopic fields (400x, Nikon Eclipse TE2000-E, Japan) and a score from 0 to 4 was given. Score 0 indicated the absence of chlamydial organisms. A score of 1 was

given when a mean of 1–5 non-replicating elementary bodies was present. Scores 2, 3 and 4 were given when a mean of 1–5, 6–10 and >10 inclusion-positive cells were observed, respectively. DNA extraction from pharyngeal swabs and subsequent nested PCR were performed as described by Van Loock et al., 2005b, demonstrating the presence of the *C. psittaci* outer membrane protein A (*ompA*) gene. Nested PCR-positive samples were further examined by a *C. psittaci ompA* genotype-specific real-time PCR (Geens et al., 2005b). Real-time PCR allowed molecular characterization of the *C. psittaci* strains involved, as well as quantification of chlamydial DNA using human or avian beta-actin housekeeping genes for normalization.

3. Results

Cell culture revealed the presence of live chlamydial organisms in both turkey groups (Table 1) and was confirmed as *C. psittaci* by nested PCR. Interestingly, *ompA* genotyping revealed the presence of three different *C. psittaci* genotypes in both groups, namely genotypes D, F and E/B with genotype D being most prevalent as shown by quantitative real-time PCR (Table 2). Although feeling healthy, the veterinary scientist was also infected as demonstrated by culture and nested PCR at t1 (Table 1). As for the turkeys, genotypes D, F and E/B were present in the human pharyngeal swab (Table 2), with genotype D being most prevalent. At that time (t1), the *C. psittaci* serum antibody titre in the veterinary scientist was 1:800.

Two weeks later (time point 2; t2), pharyngeal swab cultures of the veterinarian still revealed positive results, meaning that live organisms were still present (Table 1). Again, *C. psittaci* genotypes D, F and E/B were detected (Table 2). However, the normalised *ompA* copies of genotypes D, F and E/B for t2 were lower than for t1. Human serum taken two months later (t3) was *C. psittaci* negative. During these two

months, the veterinary scientist experienced no severe respiratory disease except for a period with mild respiratory disease being ascribed to common cold viruses, as the incident took place during the winter. The veterinarian received no treatment for psittacosis.

4. Discussion

Over the last few decades, the zoonotic nature of *C. psittaci* infections has gained a considerable amount of interest. Most psittacosis cases are diagnosed using the microimmunofluorescence (MIF) test, which is regarded as the “gold standard” in the serological diagnosis of human *C. psittaci* infections. Slides with antigens of *Chlamydia (C.) trachomatis*, *C. pneumoniae* and *C. psittaci* are used in this test and a titre of ≥ 16 is considered diagnostically significant (Smith et al., 2005). As the MIF test uses whole chlamydial organisms, serological cross-reactivity occurs between *C. psittaci* and other *Chlamydiaceae* spp. infecting humans. Using whole organisms also has the disadvantage of detecting cross-reactive antibodies to lipopolysaccharide (LPS) or proteins such as Hsp60 (Brade and Brunner, 1979; Yuan et al., 1992). A multiparametric approach with more accurate diagnostic methods to distinguish *C. psittaci* infections in humans from other chlamydial infections would be more appropriate.

In this study, *C. psittaci* zoonotic transmission was confirmed by culture followed by bacterial identification using a MAb against the *Chlamydiaceae*-specific MOMP, and by a *C. psittaci*-specific nested PCR (Van Loock et al., 2005b). The infection source was traced using a *C. psittaci ompA* genotype-specific real-time PCR (Geens et al., 2005b) revealing the presence of genotypes D, F and E/B in both turkey groups. Of course, we cannot rule out that the veterinarian infected the turkeys. However, the

person involved was not a practitioner, but a PhD student studying aMPV host cell interactions *in vitro* before starting an aMPV experiment in turkeys. So far, *C. psittaci* transmission from humans to birds has not been reported and probably occurs less frequently, as suggested by the results of Verminnen et al., (2008). These authors described a case of genotype E/B-infected humans visiting a turkey farmhouse for several days without infecting the turkeys.

As all turkeys came from the same farm we can conclude that all three genotypes were present on the farm. We do not know if all turkeys carried three different strains, as animals were not examined individually. However, we know that mixed *C. psittaci* infections do occur (Van Loock et al., 2005a).

In this case, serology could not provide additional confirmation, as the period in between the two blood samplings was too long. Antibodies found in the veterinarian at t1 could have resulted from a former *C. psittaci* infection. Moreover, although antibodies against MOMP do not cross-react with other Gram-negative bacteria, family and genus-specific epitopes on *C. psittaci* MOMP could form a target for *C. trachomatis* and/or *Cp. pneumoniae* antibodies. Thus, there is an urgent need for a *C. psittaci*-specific serological test to demonstrate human IgM and IgG antibodies.

Based on the present findings, the veterinary scientist could have become infected while transporting the turkeys to the negative pressure stables after hatching. Vertical transmission of *C. psittaci* and excretion in one-day-old turkeys has been described (Wittenbrink et al., 1993; Lublin et al., 1996; Vanrompay et al., 1997b). However, the veterinarian more likely became infected while handling and feeding the animals in the stables and during the performance of necropsies of two-week-old diseased turkeys. Necropsy was performed at the Faculty of Veterinary Medicine, in the poultry autopsy room, wetting the bird before starting but without wearing a P3

biosafety full-face mask. All autopsies are routinely performed this way, whether by
 veterinary students or by graduates in their private veterinary practice. Indeed, full
 protection for personnel and environment is only guaranteed when using class II
 biosafety cabinets, as used in research institutes. However, should we forbid
 practitioners to perform autopsies and should veterinary clinics install biosafety
 cabinets in their autopsy room? Maybe this is going too far as it would be too difficult
 to implement and enforce. The use of P3 biosafety full-face masks during autopsies
 would be a more realistic approach that could be adopted and would surely be helpful
 in preventing *C. psittaci* zoonotic transmission. It should therefore be advised or even
 made obligatory, especially when examining birds with respiratory disease.

Although several reports describe psittacosis outbreaks in humans, only few of
 them use molecular tools for strain characterization. Molecular characterization is
 currently performed by *ompA* genotyping (Geens et al., 2005b; Sachse et al., 2008),
ompA sequencing (Geens et al., 2005a) and more recently by multilocus variable
 number of tandem repeats assay (MLVA) typing (Laroucau et al., 2008).
Chlamydophila psittaci ompA genotype A, originating from infected parrots was
 defined as the outbreak strain in a veterinary teaching hospital (Heddema et al.,
 2006a). Additionally, *ompA* genotypes A, B and C could be found in hospitalized
 humans with psittacosis (Heddema et al., 2006b). However, in these cases the avian
 sources of infection could not be identified. Recently, the first report on transmission
 of *C. psittaci* genotype E/B from African grey parrots to humans was published
 (Harkinezhad et al., 2007) as well as the zoonotic transmission of genotypes A and
 E/B to psittacine pet bird owners (Vanrompay et al., 2007). However, none of these
 reports describe a simultaneous infection with three different *C. psittaci* genotypes in
 humans. It was no surprise to find *C. psittaci* genotype D most abundantly in all

samples, as genotype D is more frequently isolated from turkeys than genotypes F and E/B (Andersen, 1991; Vanrompay et al., 1997a).

In conclusion, the present study again demonstrates: 1) the zoonotic nature of the avian pathogen *C. psittaci*, 2) the need for protective recommendations, 3) the need for implementation of more accurate, sensitive and specific *C. psittaci* diagnostic tests allowing detection and strain characterization in hospital settings and health care facilities and 4) the urge for cost-effective *C. psittaci* vaccines for poultry and pet birds to reduce the zoonotic risk.

Conflict or interest statement

None of the authors (Caroline Van Droogenbroeck, Delphine S. A. Beeckman, Kristel Verminnen, Maja Marien, Hans Nauwynck, Leopold de Thibault de Boesinghe and Daisy Vanrompay) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the paper entitled “Simultaneous zoonotic transmission of *Chlamydophila psittaci* genotypes D, F and E/B to a veterinary scientist”.

Acknowledgements

The Research Foundation – Flanders (FWO – Vlaanderen) is acknowledged for providing a Ph.D. grant to Delphine S.A. Beeckman.

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1 Table 1

2 Culture and nested PCR results for turkey groups 1 and 2 and for the veterinary scientist

Sample	Culture scores ^a	Nested PCR ^b
Turkey group 1 (t1)	2	+
Turkey group 2 (t1)	2	+
Veterinary scientist (t1)	1	+
Veterinary scientist (t2)	2	+

3 ^a Scores: 0: absence of *C. psittaci*; 1: mean of 1–5 non-replicating elementary bodies; scores 2, 3 to 4:
 4 mean of 1–5, 6–10 and >10 inclusion positive cells, respectively, in five fields of view.

5 ^b + is indicative for a positive nested PCR result.

6

7

7 Table 2

8 Normalised number of *ompA* copies per 5 µl DNA extract determined by genotype-specific real-time
9 PCR

Sample	Normalised number ^a of <i>C. psittaci ompA</i> copies		
	Genotype D	Genotype F	Genotype E/B
Turkey group 1 (t1)	2,73 . 10 ⁷	1,30 . 10 ⁶	4,43 . 10 ⁵
Turkey group 2 (t1)	1,29 . 10 ⁷	3,11 . 10 ⁵	1,93 . 10 ⁵
Veterinarian (t1)	1,23 . 10 ⁴	1,54 . 10 ²	1,62 . 10 ³
Veterinarian (t2)	2,69 . 10 ³	4,60 . 10 ¹	2,54 . 10 ²

10 ^a The number of turkey or human beta-actin (ACTB) copies in the reaction was determined in order to
11 correct for inter-sample variability due to differences in sample taking and efficiency of DNA
12 extraction. Absolute *C. psittaci* copy numbers were then normalised to the amount of turkey or human
13 ACTB copies in each sample.

14