Zoonotic transmission of *Chlamydia psittaci* in a chicken and turkey hatchery

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Chlamydia psittaci is an obligately intracellular Gram-negative bacterium causing respiratory disease (chlamydiosis) or asymptomatic carriage in birds. *C. psittaci* is a zoonotic agent causing psittacosis or parrot fever in humans. Vertical and/or horizontal transmission via eggs might have serious repercussions on the *C. psittaci* infection status of poultry flocks and thus on zoonotic risk for all workers along the poultry supply chain. We therefore studied the presence of *C. psittaci* in a hatchery. In addition, we examined all ($n=4$) employees of the hatchery to evaluate the zoonotic risk. We could not detect *C. psittaci* on either eggshells or eggshell membranes. However, *C. psittaci* isolates of different outer-membrane protein A (*ompA*) genotypes were cultured from the air of both turkey (genotypes A and C) and chicken (genotype D) hatching chambers. Zoonotic transmission occurred in all employees and a mixed infection with up to three different genotypes (A, D and C), also found in air samples, was discovered. Diagnostic monitoring and reporting of *C. psittaci* infections in poultry workers should be promoted. Additionally, an efficient veterinary vaccine and information campaigns on zoonotic risk and preventive measures against *C. psittaci* transmission would be beneficial to public health.

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INTRODUCTION

Chlamydia psittaci is an obligately intracellular Gram-negative bacterium causing respiratory disease (chlamydiosis) or asymptomatic carriage in birds (Vanrompay *et al.*, 1995a). It is a zoonotic agent causing psittacosis in humans. Zoonotic transfer occurs through inhalation of contaminated dust particles or contaminated aerosols created from nasal and/or eye secretions or from dried faeces (Beeckman & Vanrompay, 2009). In humans, symptoms may include high fever accompanied by a relatively low pulse, chills, headache, myalgia, non-productive coughing and difficulty breathing. The incubation period is 5–14 days. The disease is rarely fatal in properly treated patients.

C. psittaci infections are almost endemic in poultry (Laroucau *et al.*, 2009b; Sting *et al.*, 2006; Van Loock *et al.*, 2005a; Verminnen *et al.*, 2006) and zoonotic transfer of *C. psittaci* is a threat to poultry workers all over the world (Chahota *et al.*, 2000; Dickx *et al.*, 2010; Gaede *et al.*, 2008; Laroucau *et al.*, 2009a; Verminnen *et al.*, 2008).

Vertical or transovarial transmission of *C. psittaci* during formation of the egg in the ovary/oviduct of the breeder has been described for chicken (Wittenbrink *et al.*, 1993) and turkey (Lublin *et al.*, 1996) eggs. It leads to infection of 1-day-old birds. Nevertheless, vertical transmission of *C. psittaci* is thought to be rare (Harkinezhad *et al.*, 2009). Thus, it might not be the main origin of infection for 1-day-old birds. As far as we know, horizontal infection

of embryos or other egg contents by eggshell penetrating of *C. psittaci* has not been examined. Faecal contamination of eggshells by infected hens or during egg passage in the cloaca might occur, as *C. psittaci* resides in the gut and is excreted through the faeces (Harkinezhad *et al.*, 2009). *C. psittaci* (diameter 0.2 μm) is very small compared to the well-known faecal egg contaminant *Salmonella* (diameter 0.7–1.5 μm , length 2–5 μm). Thus, *C. psittaci* could be even more easily internalized in eggs.

Vertical and/or horizontal transfer might have repercussions on the *C. psittaci* infection status of poultry flocks and thus on zoonotic risk for all workers along the poultry supply chain. We therefore studied the presence of *C. psittaci* in a hatchery. Additionally, we examined all employees of the hatchery to evaluate the zoonotic risk.

METHODS

Background. The study was conducted in a Belgian hatchery located in West Flanders. The hatchery had two hatching facilities in separate corridors: one used for both turkey and guinea fowl eggs and the other used solely for hatching chicken eggs. The hatchery was selected based on the willingness to participate in this study.

In June and September 2010, we performed a *C. psittaci* study in the turkey/guinea fowl and chicken hatching facilities, respectively. Eggshells and eggshell membranes were sampled. Additionally, *C. psittaci* bioaerosol monitoring was performed, sampling air from the turkey/guinea fowl or chicken hatching chambers. The employees, two men (M1 and M2) and two women (F1 and F2), having daily

contact with eggs and hatchlings, voluntarily participated (informed consent) in this study and provided us with a self-taken pharyngeal swab for chlamydial diagnosis.

Sampling details and processing of samples prior to analyses.

During the study in the turkey/guinea fowl and chicken facilities, at the beginning (T0), two subsequent air samples were taken from a cleaned, disinfected (formaldehyde fumigation) hatching chamber (17.12 m³; P13 Petersime, Zulte, Belgium), subsequently to be used for turkey or chicken eggs. Next, 26-day-old or 19-day-old embryonated turkey or chicken eggs, respectively, were brought in the hatching room, fumigated with formaldehyde and incubated under hatching conditions (turkey eggs, 37.4 °C and 90 % relative humidity; chicken eggs, 37 °C and 90 % relative humidity). Two days later, the hatching process started. Two subsequent air samples were taken at several time points during this hatching process: (i) in the morning when only a few animals (10 %) had hatched (T1); (ii) at noon (T2); and (iii) in the evening when most animals (90 %) had hatched (T3). Bioaerosol monitoring was performed using the MAS-100 Eco Sampler (Merck) together with the in-house-designed air collection medium ChlamyTrap at an air flow rate of 100 l min⁻¹ for 10 min (Van Droogenbroeck *et al.*, 2009a). At each time point, two samples were taken. After air sampling, Petri dishes with 20 ml ChlamyTrap were transported on ice and stored at -80 °C until tested. Samples taken at the same time were pooled for further processing. Next, 40 ml of the pooled air collection medium was divided in two equal parts. All samples were ultracentrifuged (45 000 g, 45 min, 4 °C). Pellets for culture were suspended in 500 µl chlamydia transport medium (Vanrompay *et al.*, 1992) while those for *C. psittaci* genotyping were suspended in 198 µl DNA extraction buffer (Van Look *et al.*, 2005b). Samples were stored at -80 °C until tested.

Rayon-tipped aluminium-shafted swabs (Copan; Fiers) were used to sample in twofold the employees, the eggshell of 20 randomly selected turkey (0.15 %) or chicken (0.10 %) eggs before hatching as well as the eggshell membranes of 20 additional, randomly selected turkey (0.15 %) or chicken (0.10 %) eggs after hatching. Eggs in a hatching room came from the same batch and one single parental flock. Swabs for chlamydial culture contained chlamydia transport medium, while dry swabs were used for *C. psittaci* genotyping. Swabs were transported on ice and stored at -80 °C until processed.

C. psittaci culture. The presence of viable *C. psittaci* in air samples or swabs was examined by bacterial culture in Buffalo Green Monkey (BGM) cells identifying *C. psittaci* by use of direct immunofluorescence staining at day 6 post-inoculation (IMAGEN; Oxoid) (Vanrompay *et al.*, 1994). The number of *C. psittaci*-positive cells was counted in five randomly selected microscopic fields ($\times 600$; Nikon Eclipse TE2000-E). A score from 0 to 4 was given. Score 0 indicated the absence of *C. psittaci*. Score 1 was given when a mean of 1–5 non-replicating elementary bodies were present. Scores 2, 3 and 4 were given when a mean of 1–5, 6–10 and >10 inclusion-positive cells could be observed (Vanrompay *et al.*, 1994). Subsequently, *C. psittaci* in positive samples was titrated according to the method of Spearman and Kaerber determining the log₁₀ 50 % tissue culture infective dose (TCID₅₀) per ml chlamydia transport medium or per ml ChlamyTrap air collection medium (Mayr *et al.*, 1974).

C. psittaci genotyping. For all samples, DNA extraction was performed as previously described. Outer-membrane protein A (*ompA*) genotyping was performed by a *C. psittaci* genotype-specific real-time PCR (Geens *et al.*, 2005). The test is based on using genotype-specific primers and genotype-specific TaqMan probes. Real-time PCR allowed molecular characterization of the *C. psittaci* strains involved, as well as quantification of chlamydial DNA using

the human β -actin housekeeping gene for normalization (Van Droogenbroeck *et al.*, 2009b).

RESULTS AND DISCUSSION

The results on bioaerosol monitoring and on the examination of human swabs are presented in Tables 1 and 2, respectively. *C. psittaci* was cultured from the air of both the turkey/guinea fowl and chicken hatching chambers and in both cases high titres of live organisms [up to 10^{10.75} (ml ChlamyTrap)⁻¹ for the turkey hatching chamber; up to 10^{6.25} (ml ChlamyTrap)⁻¹ for the chicken hatching chamber] were present in the air. Viable *C. psittaci* was present in hatching chambers from the start to the end of the hatching process (Table 1). Moreover, the micro-organism was even there before the hatching process started, in cleaned, disinfected hatching rooms. *C. psittaci* titres in air increased 100 to 10000 times during the hatching process of chickens or turkeys, respectively.

The hatching chamber for chickens contained *C. psittaci ompA* genotype D at all examined time points. Recently, Dickx *et al.* (2010) also found genotype D in chickens being processed in an abattoir as well as in the air of the abattoir. Genotype D is considered highly virulent and is excreted extensively (Vanrompay *et al.*, 1995b). The empty hatching chamber for turkey/guinea fowl eggs contained genotype C, and during hatching of turkeys, genotype A was found (Table 1). Thus, cleaning and disinfection (formaldehyde fumigation) of both hatching chambers after the previous egg incubation period was not sufficient to remove *C. psittaci*, as viable *C. psittaci* was still present in air samples taken at T0. Interestingly, guinea fowl eggs from France were incubated during the previous incubation period. *C. psittaci* has recently been detected by PCR in commercially raised guinea fowl in France. However, *ompA* genotyping failed (Laroucau *et al.*, 2009b). Genotype C is mostly found in ducks and geese and has been

Table 1. Presence of *C. psittaci* in air samples from the turkey/guinea fowl or chicken hatching chambers

Time point	Culture score	Titre (log ₁₀ TCID ₅₀ ml ⁻¹)	<i>ompA</i> genotype
Turkey/guinea fowl hatching chamber			
T0	4	6.75	C
T1	4	6	A
T2	4	9	A
T3	4	10.75	A
Chicken hatching chamber			
T0	4	4.25	D
T1	4	5.75	D
T2	4	5.75	D
T3	4	6.25	D

isolated from poultry workers with respiratory disease (Harkinezhad *et al.*, 2009).

Fumigation of eggs upon arrival in the hatching chamber seemed to have an effect on the amount of live organisms in the air, as genotype C was no longer detected at T1. Instead, genotype A was present, originating from turkey eggs. Genotype A has been isolated previously from turkeys (Van Loock *et al.*, 2005a; Verminnen *et al.*, 2008) and is highly virulent.

Eggshells were negative by both PCR and culture. Thus, contamination of eggs by secretions/excretions of the breeders was undetectable. Perhaps this was because only a small percentage of the eggs were sampled. However, this could also be due to the egg washing procedure performed in the hatchery upon arrival of the eggs and/or to fumigation of the eggs after being placed in the hatching chamber. On the other hand, *C. psittaci*, which is an extremely small bacterium, might rapidly penetrate the eggshell during cooling of freshly laid eggs in the breeder farm. Thus, in the future, eggshell contamination should be examined in the breeding facility instead of in a hatchery, using freshly laid eggs.

C. psittaci was not detected by either PCR or culture on eggshell membranes. Again, this could be because (i) only a small percentage of the eggs were examined or (ii) the organism was present in the animal itself, in the amnion or allantoic fluid and/or in the yolk, the yolk-sac membrane and/or the egg white. Perhaps the egg white, yolk sac and/or yolk sac membrane become infected during artificial insemination with *C. psittaci* contaminated sperm or during formation of the egg in the ovary/oviduct. This could lead to the transfer of *C. psittaci* from the egg white and/or yolk to the embryo where the organisms might stay as aberrant temporary non-reproductive bodies, otherwise the embryo would die, in cells of the intestine and/or liver. Moreover, transovarian transmission through haematogenous spread of *C. psittaci* might also occur (Vanrompay *et al.*, 1995b). Sampling egg contents and internal organs of embryos to monitor *C. psittaci* dissemination during the embryonic period could provide answers.

Nevertheless, vertical or horizontal egg contamination might be reduced by vaccinating hens and roosters on the breeder farms. However, *C. psittaci* vaccines are not available. Thus, prophylactic measures such as monitoring *C. psittaci* infections in breeders, optimal hygiene and disinfection of eggs soon after laying (Cox *et al.*, 2000) are currently the main weapons against egg contamination.

All employees ($n=4$) were *C. psittaci*-positive by culture, and mixed infections with up to three different *ompA* genotypes (A, C and D) were discovered. Previously, Van Droogenbroeck *et al.* (2009b) described a mixed *C. psittaci* genotype D, F and E/B asymptomatic infection in a veterinary surgeon. The infection originated from diseased industrial turkeys. Interestingly, the currently examined employees were also healthy. All employees had been

working in the hatching facility for more than 20 years. In the past, they all had to seek medical attention because of respiratory disease and were treated with tetracyclines without an aetiological diagnosis being performed. Thus, we cannot comment on a possible link with *C. psittaci* infections. However, as suggested by Dickx *et al.* (2010), poultry workers are almost continuously exposed to *C. psittaci* and therefore could have natural immunity against disease.

Genotype A was the most prevalent genotype in the employees, as shown by quantitative real-time PCR (Table 2). This is in accordance with the high prevalence of this genotype in air samples (Table 1). Men were less infected than women. The men spend most of their daytime in the administrative office and only assisted in the hatching chambers during 'peak moments'. The women spend most of their daytime handling both eggs and hatchlings.

Psittacosis is recognized as an occupational disease in the USA, Belgium, France, the Netherlands, Germany, Slovakia and the UK. In these countries, the occupational physician is obliged to report each case of psittacosis. In Belgium, there is a Fund for Occupational Diseases to assess *C. psittaci* cases and possibly accept them as an occupational disease, and take care of financial compensations for the employee if necessary.

Each employer is responsible for the health and safety of his employees and should focus on prevention of infections. Adequate prevention starts with a risk assessment. The employer, assisted by the occupational physician and occupational hygienist, evaluates the exposure to biological agents, taking into account the nature (contact with people or animals, or the workplace itself), intensity (the amount of infectious material handled) and duration of the worker's exposure (HSE, 2010). The risk assessment must also identify workers and other people who may not be in the workplace all the time (cleaners, maintenance and repair workers, contractors, students on placements) and members of the public who might be present (visitors) (HSE, 2010). Based on this information, adequate

Table 2. Normalized number of *C. psittaci ompA* copies per 5 µl DNA extract determined by genotype-specific real-time PCR

The number of human β -actin copies in the reaction was determined in order to correct for inter-sample variability due to differences in sample taking and efficiency of DNA extraction. Absolute *C. psittaci* copy numbers were then normalized to the number of human β -actin gene copies in each sample.

Pharyngeal sample	Genotype A	Genotype D	Genotype C
M1	3.12×10^4	1.66×10^2	Neg.
M2	1.55×10^3	1.34×10^2	Neg.
F1	3.60×10^6	3.60×10^5	1.23×10^2
F2	4.12×10^6	4.60×10^5	2.69×10^5

preventive measures can be designed. The second phase is the implementation of the preventive measures. At this stage, education and training of the employees is very important to ensure that the measures are well understood and executed. When present, a company doctor might play a crucial role in both prevention and recognition of *C. psittaci* infections.

On a personal level, prevention includes a good hand hygiene protocol, protective clothing that does not retain dust and a mouth and eye (full face) mask. It is necessary to have a transition room where protective clothing may be kept. Employees should only stay in the hatching chambers for as short a time as possible. Good environmental hygiene is also important, such as daily cleaning and disinfection of work areas and equipment, hereby preventing the creation of infectious aerosols. Some safe cleaning techniques include wet mopping of the floor with disinfectants or spraying the floor with a disinfectant or water before sweeping it. For larger areas, such as industrial hatching chambers, low-pressure washers instead of high-pressure cleaners are strongly recommended.

Conclusions

Until now, *in ovo* transmission of *C. psittaci* has been considered of minimal impact. In the present study, we found increasing amounts of *C. psittaci* in the air during hatching of turkeys or chickens. Thus, hatchlings could already be infected before arriving on the farm. Diagnostic monitoring and reporting of *C. psittaci* infections in poultry workers should be promoted. Additionally, an efficient veterinary vaccine and information campaigns on zoonotic risk and preventive measures against *C. psittaci* transmission would be beneficial to public health.

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