

# Emerging *Chlamydia psittaci* infections in chickens and examination of transmission to humans

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*Chlamydia psittaci* and atypical *Chlamydiaceae* infections are (re)-emerging in chickens. We therefore examined the prevalence of *C. psittaci*, atypical *Chlamydiaceae* and their zoonotic transmission on 19 Belgian chicken farms. Atypical *Chlamydiaceae* were not detected in chickens but 18 out of 19 farms were positive for *C. psittaci* by culture and PCR. *C. psittaci ompA* genotypes A and D were discovered. None of the examined humans ( $n=31$ ) was infected with atypical *Chlamydiaceae*, but 29 (93.5%) of them were positive for *C. psittaci* by culture and PCR. Genotypes A, D and a mixed infection with genotypes C and D were found. Humans ( $n=2$ ) working at the *C. psittaci*-negative farm never had respiratory complaints, while 25 out of 29 positive farmers (86.2%) reported yearly medical complaints potentially related to psittacosis. Four of them currently experienced respiratory disease and one of them was being treated with antibiotics. Four farmers (12.5%) mentioned that they had pneumonia after starting to keep chickens. Occupational physicians should be aware of emerging *Chlamydiaceae* infections in chickens.

Received 27 June 2013  
Accepted 7 December 2013

## INTRODUCTION

*Chlamydiaceae* are Gram-negative obligate intracellular bacteria and the species *Chlamydia psittaci* causes respiratory disease in birds. *C. psittaci* infections have been demonstrated in at least 465 different bird species, spanning 30 different bird orders (Kaleta & Taday, 2003). The symptoms may vary from inapparent to severe, depending on the chlamydial strain, stress condition, age and health status of the avian host. The symptoms in birds include rhinitis, conjunctivitis, nasal discharge, dyspnoea, diarrhoea, polyuria, anorexia, lethargy and dullness (Vanrompay *et al.*, 1995). *C. psittaci* is a well-known zoonotic agent causing psittacosis or parrot-fever in humans. During the last three decades, psittacosis outbreaks were reported in the United States (Grimes & Wyrick, 1991; Newman *et al.*, 1992), China (Ni *et al.*, 1996), India (Chahota *et al.*, 2000), Australia (Tiong *et al.*, 2007) and European poultry industries (Ryll *et al.*, 1994; Vanrompay *et al.*, 1997; Van Loock *et al.*, 2005a; Sting *et al.*, 2006; Laroucau *et al.*, 2009). Zoonotic transfer occurs through inhalation of contaminated aerosols originating from feathers, faecal material and respiratory tract exudates. Handling the plumage, carcasses and tissues of infected birds and in rare cases, mouth-to-beak contact or biting also carry a zoonotic risk (Beeckman & Vanrompay,

2009). Psittacosis in humans may vary from inapparent to fatal in untreated patients (Kováčová *et al.*, 2007). Symptoms include high fever, chills, headache, myalgia, non-productive coughing and difficult breathing (Beeckman & Vanrompay, 2009).

*C. psittaci* infections mostly occur on turkey or duck farms. However, *C. psittaci* infections are emerging in European and Asian chickens. Recently, Dickx *et al.* (2010) examined Belgian broiler breeder, broiler and layer farms by a *C. psittaci* recombinant major outer-membrane protein (MOMP)-based antibody ELISA (Verminnen *et al.*, 2006) and found 98%, 95% and 95% seropositive layers, broilers and broiler breeders, respectively. Moreover, they demonstrated *C. psittaci* genotype D in the air of chicken hatching chambers and in slaughtered Belgian and French broilers. Zoonotic transmission to hatchery and abattoir employees did occur (Dickx *et al.*, 2010; Dickx & Vanrompay, 2011), albeit without severe clinical consequences. Recently, Yin *et al.* (2013) proved Hill–Evans postulates for *C. psittaci* genotype B and D strains isolated from Belgian and French broilers.

Laroucau *et al.* (2009) detected a new atypical chlamydial agent in chickens. The atypical chicken *Chlamydiaceae* (ACC) apparently caused no disease in infected chickens, but the detection of ACC coincided with three cases of atypical pneumonia in individuals working in a French poultry abattoir. In 2012, ACC have been detected in Australian, German, Greek, Croatian, Slovenian and

Abbreviations: ACC, atypical chicken *Chlamydiaceae*; MOMP, major outer-membrane protein.

**Table 1.** *C. psittaci* culture scores

Score	Interpretation*
0	Negative (no EB, no IPC)
1	1–5 EBs
2	6–15 EBs
3	15–25 EBs and/or 1–5 IPCs
4	25–100 EBs and/or 6–15 IPCs
5	1–10 EBs per field and/or 1–5 IPCs per field

\*EB, elementary body; IPC, inclusion-positive cell.

Chinese chicken flocks (Robertson *et al.*, 2010; Zocevic *et al.*, 2012). Importantly, ACC are not detected with *C. psittaci*-specific molecular tools, rendering the need for an ACC-specific PCR. The zoonotic potential and the exact taxonomic status of ACC have yet to be defined.

The aim of the current study was to examine the presence of *C. psittaci* and ACC on Belgian chicken farms, as well as their zoonotic transmission to farmers.

## METHODS

**Study concept.** We investigated the presence of *C. psittaci* and ACC, as well as their zoonotic transmission, on 19 Belgian chicken farms: seven broiler breeder (1600 to 50 000 animals), seven broiler (200 to 150 000 animals) and five layer (7000 to 22 000 animals) farms from four different geographical regions (Antwerp, East Flanders, West Flanders and Limburg). Only one out of 19 farms kept additional bird species (ducks and geese). The study was conducted in the summer of 2012. Participating poultry farms were randomly recruited by phone. A sampling package was brought to each poultry farm and sampling was performed immediately. The package contained a questionnaire designed to assess information on: 1) the farmers' professional and non-professional activities, smoking habits, general health status, use of medication, influenza vaccination, allergies, and clinical signs potentially related to psittacosis; 2) the chicken breed, hatchery, housing, feeding, health status, medication and mortality; 3) the presence of other animals on the farm. The package also contained rayon-tipped aluminium-shafted swabs (Copan Diagnostics) for pharyngeal sampling of ten randomly selected chickens and the farmers (a maximum of two per farm). Sampling of the chickens was performed by one of the researchers. In the meantime, humans

sampled themselves at home after informed consent. Swabs for culture contained 2 ml chlamydia transport medium (Vanrompay *et al.*, 1992) while those for PCR contained 2 ml DNA stabilization buffer (Roche). Packages were transported on ice and stored at  $-80^{\circ}\text{C}$  until use.

***C. psittaci* culture.** Culture was performed using buffalo green monkey cells, and the organism was identified using the IMAGEN direct immunofluorescence assay (Oxoid) at 6 days post-inoculation (Vanrompay *et al.*, 1994). *C. psittaci*-positive cells were monitored using a CX31 fluorescence microscope with  $\times 600$  magnification (Eclipse TE2000-E; Nikon) and represented by a score ranging from 0 to 5 (Table 1).

***C. psittaci* genotyping and PCR detection of ACC.** DNA extraction of swabs was performed as described by Wilson *et al.* (1996). Briefly, specimens were centrifuged (13 000 g), suspended in 198  $\mu\text{l}$  STD buffer [0.01 M Tris/HCl (pH 8.3), 0.05 M KCl, 0.0025 M  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.5% Tween20] and 2  $\mu\text{l}$  proteinase K (20 mg  $\text{ml}^{-1}$  stock solution; Sigma-Aldrich). The specimens were incubated at  $56^{\circ}\text{C}$  for 1 h and subsequently heated at  $100^{\circ}\text{C}$  for 10 min.

A *C. psittaci*-specific nested PCR with internal inhibition control was used (Van Loock *et al.*, 2005b). Outer-membrane protein A (*ompA*) genotyping was performed by a *C. psittaci* genotype-specific real-time PCR (Geens *et al.*, 2005). The latter PCR distinguishes genotypes A to F and E/B using genotype-specific primers, genotype-specific probes and competitor oligonucleotides. Samples from chickens and humans were also examined for ACC by use of a recently developed 16S rRNA-based ACC-specific real-time PCR (Zocevic *et al.*, 2013).

**Statistics.** Potential zoonotic risk factors were statistically examined using SPSS software (SPSS). Logistic regression was used to search for non-exposure-related risk factors for *Chlamydiaceae* culture and PCR positivity. The model contained data on the information acquired from the questionnaire.

## RESULTS

### *C. psittaci* and ACC in chickens

Nineteen out of 32 chicken farms contacted participated (59%), resulting in samples from 190 chickens (10 per farm) and 31 humans (a maximum of two per farm).

ACC were not detected. Eighteen out of 19 farms (94.7%) were positive for *C. psittaci* by both culture and nested PCR (Table 2). The percentage of culture-positive chickens

**Table 2.** Pharyngeal excretion of viable *C. psittaci* by poultry ( $n=10$  per farm) and poultry workers ( $n=1$  or 2 per farm)

Farm Type	Poultry				Poultry workers			
	Farms positive/total	Culture score*		Positive % within flock	Genotype*	Culture score*		Genotype*
		Mean $\pm$ SD	Range			Mean $\pm$ SD	Range	
Broiler	7/7	1.7 $\pm$ 0.6	0–5	94	D (7/7)	1.9 $\pm$ 1.4	1–5	D (7/7)
Layer	5/5	1.8 $\pm$ 0.5	0–5	94	A (1/5); D (4/5)	2.1 $\pm$ 0.9	1–3	A (1/5); D (4/5)
Broiler Breeder	6/7	1.8 $\pm$ 0.2	1–4	100	D (6/6)	1.8 $\pm$ 0.7	1–3	D (5/6); C, D (1/6)

\*Within culture-positive farms.

**Table 3.** Viable *C. psittaci* and perceived health status in poultry farms

<i>C. psittaci</i> in broiler farms (n=10 per farm)				Health status of broilers (questionnaire)			
Age (weeks)	Positive (%)	Score (mean ± SD)	Genotype	Density (no. m <sup>-2</sup> )	Mortality (%)	Respiratory symptoms (% broods)	Antibiotic use (% broods)
2	100	2.8 ± 0.8	D	19.0	2.0	10	10 (doxy*)
<1	100	2.0 ± 1.2	D	18.0	3.5	25	0
1	60	0.9 ± 1.0	D	14.0	3.5	15	0
2–3	100	1.2 ± 0.6	D	20.0	2.8	10	10 (tylo†)
2–3	100	2.0 ± 1.3	D	10.0‡	3.0	10	0
2–3	100	1.3 ± 0.5	D	20.0	2.0	0	0
5	100	1.7 ± 0.9	D	19.5	3.0	0	0
<i>C. psittaci</i> in layer farms (n=10 per farm)				Health status of layers (questionnaire)			
32	100	1.8 ± 1.0	A	7.0	5.0	0	0
37	100	1.5 ± 0.8	D	5.0‡	NA	0	0
39	100	2.4 ± 1.0	D	9.0‡	7.0–30.0	0	0
41	100	2.1 ± 1.3	D	9.0‡	10.0	10	0
74	70	1.1 ± 1.4	D	9.0‡	4.0	0	0
<i>C. psittaci</i> in broiler breeder farms (n=10 per farm)				Health status of broiler breeders (questionnaire)			
2	100	1.4 ± 0.8	D	10.0	2.0	100	0
31	0	0.0 ± 0.0		7.0	NA	0	0
34	100	2.1 ± 1.2	D	16.5	5.0–10.0	0	0
42	100	2.0 ± 0.9	D	7.2	10.0	10	0
48	100	1.8 ± 1.0	D	6.5	9.3	0	0
50	100	1.6 ± 0.5	D	NA	1.5	0	0
50	100	1.9 ± 1.0	D	9.0	1.2	10	10 (doxy*)

NA, Not available.

\*Doxycycline.

†Tylosin.

‡Chickens have access to outside area.

**Table 4.** *C. psittaci*: perceived health status and psittacosis-compatible symptoms in farm employees

Broiler farm employees													
Viable <i>C. psittaci</i>		Personnel data			Current health status		Yearly medical complaints†					Confirmed pneumonia (no. of years ago)	
Score	Genotype	Period	Time (h day <sup>-1</sup> except for *)	Aves at home	Current symptoms	Antibiotic treatment	Fl	Re	GI	Ey	De		
Male	5	D	27 years	2*	–	–	–	F <sup>1</sup> ‡, M <sup>1</sup>	NPC <sup>1</sup>	S <sup>1</sup> , D <sup>1</sup>	–	–	–
	1	D	20 years	7	Layers	–	–	–	–	–	–	–	–
	1	D	2 years	7	Birds	–	–	F <sup>1</sup> , M <sup>2</sup>	–	–	–	–	–
	1	D	15 years	2	Layers	–	–	F <sup>1</sup> , M <sup>1</sup>	NPC <sup>2</sup>	–	–	–	–
	1	D	12 years	1	–	–	–	M <sup>2</sup>	–	–	–	R <sup>2</sup>	3
	2	D	20 years	1	–	–	–	F <sup>1</sup> , M <sup>3</sup>	–	V <sup>1</sup>	E <sup>1</sup>	–	–
	1	D	30 years	2	–	–	–	–	PC <sup>3</sup>	–	–	–	19
Female	4	D	25 years	8	–	–	–	F <sup>3</sup> , M <sup>3</sup>	PC <sup>1</sup>	B <sup>1</sup> , D <sup>3</sup>	–	R <sup>1</sup>	–
	3	D	13 years	3	–	–	–	F <sup>2</sup> , M <sup>2</sup>	NPC <sup>2</sup>	–	–	–	2 (pleuritis)
	1	D	30 years	7	–	Cold	–	–	–	–	–	–	–
Every production round a cold at ± 5 weeks													
Broiler breeder employees													
Male	2	D	15 years	2	–	–	–	–	NPC <sup>2</sup>	–	–	–	–
	1	D	7 years	1	–	–	–	F <sup>1</sup> , M <sup>1</sup>	PC <sup>1</sup>	V <sup>1</sup> , S <sup>1</sup> , D <sup>1</sup>	–	–	–
	2	D	19 years	3	–	–	–	F <sup>1</sup> , M <sup>1</sup>	NPC <sup>1</sup>	S <sup>1</sup>	–	–	–
	1	D	4.5 years	8	–	Cold	–	F <sup>1</sup>	NPC <sup>2</sup> , B <sup>2</sup>	–	E <sup>2</sup>	–	–
	1	D	27 years	4	–	–	–	–	–	–	–	–	22
	2	D	25 years	8	–	–	–	F <sup>2</sup> , M <sup>2</sup>	PC <sup>2</sup> , B <sup>2</sup>	V <sup>2</sup> , S <sup>2</sup> , D <sup>2</sup>	E <sup>2</sup>	–	–
	0	–	2 years	1	–	–	–	–	–	–	–	–	–
	0	–	17 years	3	–	–	–	–	–	–	–	–	–
Female	3	D	15 years	2	–	'Allergic feeling'	–	T <sup>3</sup>	NPC <sup>2</sup>	–	–	R <sup>1</sup>	–
	2	D	7 years	2	–	–	–	F <sup>1</sup> , M <sup>2</sup>	PC <sup>2</sup>	V <sup>1</sup> , S <sup>1</sup> , D <sup>1</sup>	–	R <sup>1</sup>	–
	2	D	19 years	4	–	Cold	–	–	NPC <sup>1</sup>	S <sup>1</sup>	–	–	–
	1	D	27 years	4	–	–	–	–	–	–	–	–	–
	3	D + C	30 years	8	–	–	–	F <sup>2</sup> , M <sup>2</sup>	PC <sup>2</sup>	V <sup>2</sup> , S <sup>2</sup> , D <sup>1</sup>	–	–	–
								Augmentin (4 weeks ago)					

**Table 4.** cont.

Layer farm employees												
Viable <i>C. psittaci</i>		Personnel data			Current health status		Yearly medical complaints†					Confirmed pneumonia (no. of years ago)
Score	Genotype	Period	Time (h day <sup>-1</sup> except for *)	Aves at home	Current symptoms	Antibiotic treatment	Fl	Re	GI	Ey	De	
Male	3	D	40 years	1	–	–	–	F <sup>1</sup> , M <sup>2</sup>	NPC <sup>1</sup> , DB <sup>1</sup>	–	–	–
	3	D	7 years	5	–	–	–	M <sup>2</sup>	NPC <sup>1</sup>	–	–	–
	1	D	12 years	0.5 *	–	–	–	–	NPC <sup>3</sup> , Ex <sup>3</sup>	–	–	–
	2	D	2 months	0.5 *	Ducks, geese	Cold	–	F <sup>1</sup>	–	–	–	–
	3	A	17 years	3	–	–	–	F <sup>2</sup>	–	S <sup>2</sup> , D <sup>2</sup>	–	R <sup>2</sup>
Female	3	D	24 years	3	–	–	–	F <sup>1</sup> , M <sup>3</sup>	NPC <sup>1</sup> , B <sup>1</sup>	–	–	–
	1	D	23 years	4	–	–	–	–	–	–	–	–
	1	NA	3 years	3	–	–	–	F <sup>2</sup> , M <sup>2</sup>	–	–	E <sup>2</sup>	–

NA, not applicable.

\*Hours week<sup>-1</sup>.

†Fl, Flu-like; F, fever; M, myalgia; T, tired-fatigue; Re, respiratory; NPC or PC, (non-) productive cough; B, painful breathing; Ex, morning expectoration; GI, gastrointestinal; V, vomiting; D, diarrhoea; S, stomach ache; Ey, eye; E, painful eyes; De, dermatologic; R, non-specific rash.

‡1, Once or twice; 2, repeatedly; 3, frequently.

per farm varied from 60–100%. *C. psittaci* genotype D was present in 17/18 (94.4%) of positive farms, while a genotype A infection was discovered in 1/18 positive farms (Table 3). Thus, *C. psittaci* was found in broiler breeders, broilers and layers. According to the questionnaire, respiratory symptoms were present in infected broiler breeders (3/7 farms; 42.8%), infected broilers (5/7 farms; 71.4%) and infected layers (1/5 farms; 20%). Mean mortality for infected broiler breeders, broiler and layer farms was 5.4%, 2.8% and 9.8%, respectively. One out of six infected broiler breeder and 2/7 infected broiler farms currently used antibiotics [tylosin (Pharmasin; Eurovet) and doxycycline (Soludox; Eurovet)]. Nevertheless, we were able to detect viable *C. psittaci*. A high stocking density (number of chickens  $m^{-2}$ ) was significantly related to the risk of acquiring chlamydiosis ( $P=0.006$ ). The negative farm was the only one with no poultry farms nearby ( $<4$  km). Plus, it was the only farm with a very long sanitary period (8 weeks), which is the period in-between emptying the barn, cleaning, disinfecting and restocking (usually 1–2 weeks). However, the latter two observations were not significantly related to the risk of chlamydiosis in chickens ( $P=0.08$  and  $0.157$ , respectively). Antibiotics were not used at the time of sampling.

### Zoonotic transmissions

The study population consisted of 11 women and 20 men and the average age was 42 years. Three of the 31 farmers (9.6%) were vaccinated against human influenza. None were infected by ACC. However, 29/31 (93.5%) of humans were *C. psittaci*-positive by both culture and the *C. psittaci*-specific nested PCR. *C. psittaci* genotype D ( $n=26$ ), genotype A ( $n=1$ ) and a mixed genotype D plus C infection ( $n=1$ ) were discovered in farmers. Genotyping revealed no result for one sample. This sample originated from a female employee of a layer farm which only kept chickens (Table 4). Thus, *C. psittaci* zoonotic transmission was detected on all but one examined chicken farm.

Many *C. psittaci* positives were found, but only four of them (13.7%), who were non-smokers and had no allergies, currently experienced respiratory diseases (coughing,  $n=3$  and/or rhinitis,  $n=1$ ; sinusitis,  $n=1$ ; severe bronchitis,  $n=1$ ). They were all infected with genotype D, and the person with bronchitis was currently treated with Augmentin (Glaxo Smith Kline). We informed the farmers and their physicians of the diagnostic results.

Humans ( $n=2$ ) working on the *C. psittaci*-negative farm never had respiratory complaints, while 25/29 positive farmers (86.2%) reported yearly medical complaints potentially related to psittacosis (Table 4). Four out of 31 farmers (12.5%) mentioned that they had pneumonia after starting to keep chickens (Table 4).

No potential risk factor like age, gender, living in the direct environment of the farm, number of years employed in the sector, daily time in contact with chickens, pet animals,

smoking behaviour or medical complaints was significantly related to psittacosis.

## DISCUSSION

We examined the occurrence of *C. psittaci* on 19 Belgian chicken farms, as well as zoonotic transmissions of these pathogens to farmers because *C. psittaci* is now (re)-emerging in chickens. Limited reports from 1960 to 2000 suggest that chickens are less sensitive to *C. psittaci* infections. However, during the last decade, *C. psittaci* has been detected and isolated from chickens raised in Australia, Belgium, China, France and Germany (Yang *et al.*, 2007; Gaede *et al.*, 2008; Zhang *et al.*, 2008; Laroucau *et al.*, 2009; Robertson *et al.*, 2010; Zhou *et al.*, 2010; Dickx & Vanrompay, 2011). Recently, Yin *et al.* (2013) proved Hill–Evans postulates for *C. psittaci* genotype B and D strains isolated from Belgian and French broilers. Less is known about *C. psittaci* genotypes infecting chickens. Up to now, genotypes B, C, D, F and E/B have been found in chickens (Gaede *et al.*, 2008; Zhang *et al.*, 2008; Dickx *et al.*, 2010; Zhou *et al.*, 2010; Yin *et al.*, 2013).

*C. psittaci* is apparently not the only emerging chlamydial pathogen in chickens. Laroucau *et al.* (2009) discovered a new chlamydial agent in chickens raised in France, designated atypical chicken *Chlamydiaceae* (ACC). Remarkably, ACC-positive chickens appeared healthy, but the discovery of ACC coincided with three cases of atypical pneumonia in French poultry workers (Laroucau *et al.*, 2009), warranting the need for epidemiological surveillance in chickens. Since then, ACC has been found in chickens raised in China, Croatia, Germany, Greece and Slovenia (Zocevic *et al.*, 2012). This is why we also included the recently developed ACC-specific real-time PCR in our epidemiological study.

*C. psittaci* was highly prevalent in chickens and humans. *OmpA* genotyping revealed the presence of genotypes A, C, and especially D. To our knowledge, this is the first time that genotype A, the second time that genotype C, and only the third time that genotype D has been identified in chickens. Genotype A is most often found in *Psittaciformes* (cockatoos, parrots, parakeets and lorries) and is frequently transmitted from pet birds to humans. Genotype A has also been isolated from turkeys and wild birds (Van Loock *et al.*, 2005; Verminnen *et al.*, 2006; Geigenfeind *et al.*, 2011; Kalmar *et al.*, 2013). Thus, the pathogen is not restricted to *Psittaciformes* and was probably never noticed before in chickens. However, genotypes B and D seem to be most prevalent in chickens. Genotype D is most often found in turkeys, but recently has been associated with zoonotic transfer from chickens to slaughterhouse employees (Dickx *et al.*, 2010). Genotype C has primarily been isolated from ducks and geese, but has been found once before in chickens, namely in China (Zhang *et al.*, 2008).

ACC were not detected in chickens, suggesting that ACC are currently not widespread in Belgian chicken flocks, at

least when compared with *C. psittaci*. However, we cannot exclude the absence of this emerging chlamydial agent in our chicken flocks. Respiratory disease was present, albeit not on all *C. psittaci*-infected farms. Respiratory disease was most frequently present on broiler farms, followed by broiler breeder and layer farms. Only broiler and broiler breeder farms claimed to use antibiotics [tylosin (Pharmasin; Eurovet) and doxycycline (Soludox; Eurovet)]. Antibiotic usage in European poultry has decreased over the last few years (Moulin *et al.*, 2008) (BelVet-SAC report 2012: <http://www.belvetsac.ugent.be/>), but antibiotics are still frequently used without a proper diagnosis; among these are the ones active against *C. psittaci*, with the risk of creating tetracycline resistance as occurred for *Chlamydia suis* (Dugan *et al.*, 2004).

Interestingly, a high stocking density (number of chickens  $m^{-2}$ ) was the only risk factor that was positively correlated with the occurrence of *C. psittaci* in chickens. This finding was no surprise, as *C. psittaci* transmission most often occurs from one bird to another bird close by.

As for chickens, ACC were not detected in farmers. However, viable *C. psittaci* were present in 93.5% of the farmers. Genotypes A, C and, as in chickens, especially genotype D were discovered in the farmers. In our study, genotype C [most frequently found in *Anseriformes* (ducks and geese)] was not detected in chickens, but we cannot exclude the absence of genotype C on the farms, as only 10 chickens were sampled. Zoonotic transmissions of genotypes A, C and D, and even mixed genotype A, C and D infections in poultry workers, have been observed before by Dickx & Vanrompay (2011), examining employees of a turkey and chicken hatchery. Thus, *C. psittaci*-infected chickens present a substantial zoonotic risk. One human sample could not be genotyped, which could indicate the presence of a new genotype. Attempts to grow the strain to a higher bacterial titre for *ompA* sequencing failed.

Humans ( $n=2$ ) from the *C. psittaci*-negative farm never had respiratory complaints, while 25/29 (86.2%) of humans, all working on *C. psittaci*-positive farms, reported yearly medical complaints potentially related to psittacosis (Table 4). Four out of 31 farmers (12.5%) mentioned in the questionnaire that they had pneumonia after starting to keep chickens, which was higher than the yearly rate of 8 in 1000 pneumonia cases in Belgium. It is likely that chicken farmers are regularly infected, creating immunity which protects them against severe disease. However, yearly complaints about fever and respiratory disease were of interest (Table 4). Whether farmers become carriers, the clinical consequences and the importance of co-infections with other human respiratory pathogens are unknown.

Preventing avian chlamydiosis in poultry is difficult because of the endemic nature of the bacteria, the long-term survival of the bacteria in organic material, the intermittent shedding and the many asymptomatic carriers (Pelle-Duporte & Gendre, 2001). An all-in, all-out rearing regime, with thorough cleaning and disinfecting between

broods is obligatory. *C. psittaci* is highly susceptible to heat and disinfectants (quaternary ammonium compounds, household bleach) but is resistant to drying, acids and alkalis (Smith *et al.*, 2005). Access of wild birds to the animals or food should be prevented. Equipment should be regularly cleaned and disinfected when used for several barns at the farm.

Personal protective measures involve a good hand hygiene protocol and protective clothing, including gloves and an air filter full-face mask. A transition room should be available where protective clothing may be kept. The two most important collective protective measures are ventilation and cleaning. Natural or mechanical ventilation should try to prevent aerosol accumulation and cross-contamination between the different barns. Even continuous disinfection (although expensive) of the air in the barns could be considered. Education and training are very important to guarantee that the preventive measures are well understood and performed (Deschuyffeleer *et al.*, 2012).

## Conclusions

Despite governments' obligation to assess any biohazard in the workplace, knowledge on *C. psittaci* and especially ACC in chickens is still relatively undeveloped and a specific risk assessment in poultry production remains to be established. Many health care providers are not familiar with psittacosis, especially with its occupational and zoonotic character. An occupational physician assigned to modern, vertically integrated poultry farming, covering the complete poultry production ranging from the feeding mill to processing facilities, could conduct a campaign to raise general awareness and to inform poultry workers on collective and personal protective measures. The occupational physician should address local physicians with a written document, as this may lead to an early diagnosis and treatment in poultry workers (Deschuyffeleer *et al.*, 2012). However, most benefit is to be expected from an efficient avian *Chlamydia* vaccine.

## ACKNOWLEDGEMENTS

The study was funded by Ghent University (grant IOF10/STEP/002) and by MSD Animal Health, Boxmeer, The Netherlands. Annelien Dumont and Ellen Audenaert are acknowledged for technical assistance. L. Braeckman (Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University) is acknowledged for providing the medical questionnaire. We gratefully thank Debby Braeckmans and Geert Van Den Abeele for distributing sampling packages to the farms.

## REFERENCES

- Beeckman, D. S. & Vanrompay, D. C. (2009). Zoonotic *Chlamydo-phil* *psittaci* infections from a clinical perspective. *Clin Microbiol Infect* **15**, 11–17.

- Chahota, R., Katoch, R. C., Singh, S. P., Verma, S. & Mahajan, A. (2000). Concurrent outbreak of chlamydiosis and aflatoxicosis among chickens in Himachal Pradesh, India. *Veterinarski Arhiv* **70**, 207–213.
- Deschuyffeleer, T. P., Tyberghien, L. F., Dickx, V. L., Geens, T., Saelen, J. M., Vanrompay, D. C. & Braeckman, L. A. (2012). Risk assessment and management of *Chlamydia psittaci* in poultry processing plants. *Ann Occup Hyg* **56**, 340–349.
- Dickx, V. & Vanrompay, D. (2011). Zoonotic transmission of *Chlamydia psittaci* in a chicken and turkey hatchery. *J Med Microbiol* **60**, 775–779.
- Dickx, V., Geens, T., Deschuyffeleer, T., Tyberghien, L., Harkinezhad, T., Beeckman, D. S., Braeckman, L. & Vanrompay, D. (2010). *Chlamydia psittaci* zoonotic risk assessment in a chicken and turkey slaughterhouse. *J Clin Microbiol* **48**, 3244–3250.
- Dugan, J., Rockey, D. D., Jones, L. & Andersen, A. A. (2004). Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial *inv*-like gene. *Antimicrob Agents Chemother* **48**, 3989–3995.
- Gaede, W., Reckling, K. F., Dresenkamp, B., Kenkies, S., Schubert, E., Noack, U., Irmischer, H. M., Ludwig, C., Hotzel, H. & Sachse, K. (2008). *Chlamydia psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. *Zoonoses Public Health* **55**, 184–188.
- Geens, T., Dewitte, A., Boon, N. & Vanrompay, D. (2005). Development of a *Chlamydia psittaci* species-specific and genotype-specific real-time PCR. *Vet Res* **36**, 787–797.
- Grimes, J. E. & Wyrick, P. B. (1991). Chlamydiosis (Ornithosis). In *Diseases of Poultry*, pp. 311–325. Edited by B. W. Clenk, H. J. Barnes, C. W. Beard, W. M. Reid & H. W. Yoder. Ames, IA: Iowa State University Press.
- Kaleta, E. F. & Taday, E. M. (2003). Avian host range of *Chlamydia* spp. based on isolation, antigen detection and serology. *Avian Pathol* **32**, 435–462.
- Kováčová, E., Majtán, J., Botek, R., Bokor, T., Blaskovicová, H., Solavová, M., Ondicová, M. & Kazár, J. (2007). A fatal case of psittacosis in Slovakia, January 2006. *Euro Surveill* **12**, E070802.1.
- Laroucau, K., Vorimore, F., Aaziz, R., Berndt, A., Schubert, E. & Sachse, K. (2009). Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. *Infect Genet Evol* **9**, 1240–1247.
- Moulin, G., Cavalié, P., Pellanne, I., Chevance, A., Laval, A., Millemann, Y., Colin, P., Chauvin, C. on behalf of the Antimicrobial Resistance *ad hoc* Group of the French Food Safety Agency (2008). A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. *J Antimicrob Chemother* **62**, 617–625.
- Newman, C. P., Palmer, S. R., Kirby, F. D. & Caul, E. O. (1992). A prolonged outbreak of ornithosis in duck processors. *Epidemiol Infect* **108**, 203–210.
- Ni, A. P., Lin, G. Y., Yang, L., He, H. Y., Huang, C. W., Liu, Z. J., Wang, R. S., Zhang, J. S., Yu, J. Y. & other authors (1996). A seroepidemiologic study of *Chlamydia pneumoniae*, *Chlamydia trachomatis* and *Chlamydia psittaci* in different populations on the mainland of China. *Scand J Infect Dis* **28**, 553–557.
- Pelle-Duporte, D. & Gendre, I. (2001). Ornithosis epidemics in a poultry slaughterhouse. *INRS, Documents for the occupational physician* **85**, 49–57.
- Robertson, T., Bibby, S., O'Rourke, D., Belfiore, T., Agnew-Crumpton, R. & Noormohammadi, A. H. (2010). Identification of Chlamydial species in crocodiles and chickens by PCR-HRM curve analysis. *Vet Microbiol* **145**, 373–379.
- Ryll, M., Hinz, K. H., Neumann, U. & Behr, K. P. (1994). Pilotstudie über das Vorkommen von *Chlamydia psittaci*-Infektionen in kommerziellen Putenherden Niedersachsens. *Dtsch Tierärztl Wochenschr* **101**, 163–165.
- Smith, K. A., Bradley, K. K., Stobierski, M. G., Tengelsen, L. A. & National Association of State Public Health Veterinarians Psittacosis Compendium Committee (2005). Compendium of measures to control *Chlamydia psittaci* (formerly *Chlamydia psittaci*) infection among humans (psittacosis) and pet birds, 2005. *J Am Vet Med Assoc* **226**, 532–539.
- Sting, R., Lerke, E., Hotzel, H., Jodas, S., Popp, C. & Hafez, H. M. (2006). Vergleichende Untersuchungen zum Nachweis von *Chlamydia psittaci* und *Chlamydia abortus* in Putenmastbetrieben mittels Zelikultur, ELISA und PCR. *Dtsch Tierärztl Wochenschr* **113**, 50–54.
- Tiong, A., Vu, T., Counahan, M., Leydon, J., Tallis, G. & Lambert, S. (2007). Multiple sites of exposure in an outbreak of ornithosis in workers at a poultry abattoir and farm. *Epidemiol Infect* **135**, 1184–1191.
- Van Loock, M., Geens, T., De Smit, L., Nauwynck, H., Van Empel, P., Naylor, C., Hafez, H. M., Goddeeris, B. M. & Vanrompay, D. (2005a). Key role of *Chlamydia psittaci* on Belgian turkey farms in association with other respiratory pathogens. *Vet Microbiol* **107**, 91–101.
- Van Loock, M., Verminnen, K., Messmer, T. O., Volckaert, G., Goddeeris, B. M. & Vanrompay, D. (2005b). Use of a nested PCR-enzyme immunoassay with an internal control to detect *Chlamydia psittaci* in turkeys. *BMC Infect Dis* **5**, 76.
- Vanrompay, D., Ducatelle, R. & Haesebrouck, F. (1992). Diagnosis of avian chlamydiosis: specificity of the modified Giménez staining on smears and comparison of the sensitivity of isolation in eggs and three different cell cultures. *Zentralbl Veterinarmed B* **39**, 105–112.
- Vanrompay, D., Van Nerom, A., Ducatelle, R. & Haesebrouck, F. (1994). Evaluation of five immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens from turkeys. *J Clin Microbiol* **32**, 1470–1474.
- Vanrompay, D., Ducatelle, R. & Haesebrouck, F. (1995). *Chlamydia psittaci* infections: a review with emphasis on avian chlamydiosis. *Vet Microbiol* **45**, 93–119.
- Vanrompay, D., Butaye, P., Van Nerom, A., Ducatelle, R. & Haesebrouck, F. (1997). The prevalence of *Chlamydia psittaci* infections in Belgian commercial turkey poults. *Vet Microbiol* **54**, 85–93.
- Verminnen, K., Van Loock, M., Hafez, H. M., Ducatelle, R., Haesebrouck, F. & Vanrompay, D. (2006). Evaluation of a recombinant enzyme-linked immunosorbent assay for detecting *Chlamydia psittaci* antibodies in turkey sera. *Vet Res* **37**, 623–632.
- Wilson, P. A., Phipps, J., Samuel, D. & Saunders, N. A. (1996). Development of a simplified polymerase chain reaction-enzyme immunoassay for the detection of *Chlamydia pneumoniae*. *J Appl Bacteriol* **80**, 431–438.
- Yang, J., Yang, Q., Yang, J. & He, C. (2007). Prevalence of avian *Chlamydia psittaci* in China. *Bulletin of the Veterinary Institute in Pulawy* **51**, 347–350.
- Yin, L., Kalmar, I. D., Lagae, S., Vandendriessche, S., Vanderhaeghen, W., Butaye, P., Cox, E. & Vanrompay, D. (2013). Emerging *Chlamydia psittaci* infections in the chicken industry and pathology of *Chlamydia psittaci* genotype B and D strains in specific pathogen free chickens. *Vet Microbiol* **162**, 740–749.
- Zhang, F., Li, S., Yang, J., Pang, W., Yang, L. & He, C. (2008). Isolation and characterization of *Chlamydia psittaci* isolated from laying hens with cystic oviducts. *Avian Dis* **52**, 74–78.
- Zhou, J., Qiu, C., Lin, G., Cao, X. & Zheng, F. (2010). Isolation of *Chlamydia psittaci* from laying hens in China. *Vet Res* **3**, 43–45.
- Zocevic, A., Vorimore, F., Marhold, C., Horvatek, D., Wang, D., Slavec, B., Prentza, Z., Stavianis, G., Prukner-Radovcic, E. & other



**authors (2012).** Molecular characterization of atypical *Chlamydia* and evidence of their dissemination in different European and Asian chicken flocks by specific real-time PCR. *Environ Microbiol* **14**, 2212–2222.

**Zocecic, A., Vorimore, F., Vicari, N., Gasparini, J., Jacquin, L., Sachse, K., Magnino, S. & Laroucau, K. (2013).** A real-time PCR assay for the detection of atypical strains of *Chlamydiaceae* from pigeons. *PLoS ONE* **8**, e58741.