

Outbreak of psittacosis in a group of women exposed to *Chlamydia psittaci*-infected chickens

K Laroucau (Karine.laroucau@anses.fr)^{1,2}, R Aaziz^{1,2}, L Meurice³, V Servas³, I Chossat⁴, H Royer⁵, B de Barbeyrac⁶, V Vaillant⁷, J L Moyen⁸, F Meziani⁹, K Sachse¹⁰, P Rolland³

1. Anses, Laboratoire de Santé Animale, Unité Zoonoses Bactériennes, Laboratoire National de Référence pour la Chlamydiose Aviaire, Maisons-Alfort, France
2. These authors contributed equally to the work
3. Institut de Veille Sanitaire (InVS), Département de Coordination des Alertes et des Régions, Cellule de l'InVS en région Aquitaine, Bordeaux, France
4. Unité Médecine Interne et Infectiologie, Centre Hospitalier de Villeneuve sur Lot, Villeneuve sur Lot, France
5. Agence Régionale de Santé Aquitaine – Délégation territoriale du Lot-et-Garonne, Pau, France
6. Université Bordeaux, Centre National de Référence des Chlamydiae, Bordeaux, France
7. Institut de Veille Sanitaire, Saint-Maurice, France
8. Laboratoire Départemental d'Analyse et de Recherche de Dordogne, Coulounieix-Chamiers, France
9. Direction Départementale de la Cohésion Sociale et de la Protection des Populations de Lot-et-Garonne, Agen, France
10. Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), OIE Reference Laboratory for Chlamydiosis, Jena, Germany

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Eight cases of psittacosis due to *Chlamydia psittaci* were identified in May 2013 among 15 individuals involved in chicken gutting activities on a mixed poultry farm in France. All cases were women between 42 and 67 years-old. Cases were diagnosed by serology and PCR of respiratory samples. Appropriate treatment was immediately administered to the eight hospitalised individuals after exposure to birds had been discovered. In the chicken flocks, mainly *C. gallinacea* was detected, a new member of the family *Chlamydiaceae*, whereas the ducks were found to harbour predominantly *C. psittaci*, the classical agent of psittacosis. In addition, *C. psittaci* was found in the same flock as the chickens that the patients had slaughtered. Both human and *C. psittaci*-positive avian samples carried the same *ompA* genotype E/B of *C. psittaci*, which is widespread among French duck flocks. Repeated grassland rotations between duck and chicken flocks on the farm may explain the presence of *C. psittaci* in the chickens. Inspection by the veterinary service led to temporary closure of the farm. All birds had to be euthanised on site as no slaughterhouses accepted processing them. Farm buildings and grasslands were cleaned and/or disinfected before the introduction of new poultry birds.

Introduction

The members of the family *Chlamydiaceae* are Gram-negative obligate intracellular bacteria with a unique biphasic developmental cycle. Avian chlamydiosis, also called psittacosis, is a zoonosis caused by *Chlamydia psittaci*. More than 467 avian species can be affected by chlamydial infections [1]. In birds, clinical

signs vary greatly in severity and depend on the species and age of the bird as well as the infecting strain involved. Zoonotic transmission mainly occurs via inhalation of infected excretions and discharges [2,3]. The spectrum of clinical manifestations in humans is wide and varies considerably, from inapparent to a mild influenza-like illness or serious atypical pneumonia, with occasionally fatal outcome [4]. Intermittent shedding by animal carriers represents an important path of infection for birds and humans. Avian strains of *C. psittaci* are currently divided into 13 genotypes of the outer membrane protein A (*OmpA*), designated A to F, E/B, 1V, 6N, Mat16, R54, YP84, CPX0308 [5]. A degree of host specificity can be noted, with genotype A being detected mostly in psittacines, B and E in pigeons, or C and E/B in ducks [6].

In domesticated birds, *C. psittaci* infections occur most commonly in turkeys and ducks. Recent studies reported frequent *C. psittaci* infections in European and Asian chickens [7-9]. While *C. psittaci* was until recently considered to be the sole causative agent of avian chlamydiosis, two new avian species, *C. avium* and *C. gallinacea*, have recently been described [10]. Based on currently available data, using both broad-range and specific diagnostic tools, it seems likely that *C. gallinacea* is widely disseminated among poultry and *C. avium* is frequently found in pigeons. PCR-based tools have been developed for their specific detection [11,12]. The aetiological importance of these new agents in humans or birds is at present not well understood.

TABLE 1

Diagnostic data and background information of psittacosis cases, France, May 2013 (n = 8)

Case	Day of first clinical signs	Day of hospitalisation	Clinical diagnosis / symptoms	Day of discharge	Serology		PCR <i>C. psittaci</i> (incA)				Case status	Participation in meal preparation	Underlying diseases
					Date	Result	Date	Material	Result	Geno typing			
1	24 May	26 May	Pneumonia	4 Jun	30 May	Negative	29 May	Sputum	Positive	NA	Confirmed	Both sessions	Type 2 diabetes
2	24 May	26 May	Fever	4 Jun	29 May 11 Jun	IgG 1/128 Negative	29 May	Sputum	Positive	NA	Confirmed	Both sessions	Rheumatoid arthritis
3	25 May	27 May	Pneumonia	6 Jun	30 May	Negative	3 Jun	Throat swab ^a	Negative	ND	Possible	Both sessions	None
4	25 May	27 May	Pneumonia	5 Jun	30 May ND	Negative ND	29 May	Sputum	Negative	ND	Possible	Both sessions	Type 2 diabetes
5	25 May	29 May	Fever, cough	6 Jun	10 Jun	Negative	29 May	Throat swab ^a	Negative	ND	Confirmed	Both sessions	None
6	26 May	29 May	Fever	6 Jun	30 May	Negative	29 May	Sputum	Positive	E/B - 859	Confirmed	Both sessions	Asthma
7	27 May	30 May	Fever, cough	5 Jun	30 May	IgG 1/64	3 Jun	Throat swab ^a	Negative	ND	Probable	First session	Cirrhosis
8	28 May	29 May	Pneumonia	6 Jun	29 May	Negative	ND	ND	ND	ND	Possible	Both sessions	None

NA: no amplification; ND: not done.

^a Taken after the onset of the treatment.

In France, *C. psittaci* genotypes C and E/B are highly prevalent in duck flocks [13], and human cases linked to this species are not rare. The French reference centre for psittacosis, which provides passive surveillance, diagnosed 32 cases in 2012–13. For 17 of them, exposure to ducks could be clearly established. In the present paper, we report an outbreak of psittacosis due to *C. psittaci* in women who gutted chickens bred on a farm where also ducks were raised.

Methods

Epidemiological investigations

Case definition

In the present study, a patient with fever and/or respiratory symptoms who participated in the evisceration of chickens on 14 and/or 24 May was regarded as a possible case. A probable case was a possible case combined with an IgG titre >32. A confirmed case was a possible case with either positive detection of *C. psittaci* by PCR in a respiratory sample, or seroconversion, or a fourfold increase in IgG titre.

Questionnaire

After notification of a cluster of psittacosis cases to the public health authorities of the Department of Aquitaine, a telephone investigation was conducted. A questionnaire covering age, sex, date of onset of clinical signs, symptoms and travel activities within 15 days before illness onset was completed for each hospitalised person after they were discharged.

Microbiological investigations

Human samples

Aliquots of early serum from each patient were sent to the National Reference Centre for Chlamydiae (NRC, Bordeaux, France). Sputum samples from five patients were collected during their hospitalisation, as well as throat swabs from four patients after medication.

Direct detection of *Chlamydia psittaci* from human samples

Clinical samples were extracted by using the automated MagNA Pure DNA extraction (Roche Diagnostics, Meylan, France) [14] then analysed using a *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene [15] and a specific *incA* real-time PCR protocol [16].

Serology

A commercialised micro-immunofluorescence test was used (Chlamydia MIF, Focus, Eurobio, France). This assay can distinguish between IgM and IgG subclasses. Each well contained four spots: one yolk sac control and three individual antigen spots consisting of elementary bodies of *C. psittaci*, *C. trachomatis* and *C. pneumoniae* suspended in a yolk sac matrix. Each run included a positive (murine hyperimmune serum) and negative control (human serum). For IgG, the serum

was serially diluted from 1/16. The reciprocal of the highest serum dilution yielding apple-green fluorescence was termed the serum endpoint titre. For IgM, only one serum dilution was tested (1/16) and the result was assessed qualitatively, i.e. positive or negative.

Traceback investigations

Animal samples

To identify the source of infection of the patients, a survey was conducted in all poultry flocks of the farm. On 10 June, samples were collected from all duck flocks ($n=4$, denominated MD for mule duck) and chicken flocks ($n=3$, denominated BC for broiler chicken) on the farm. In each sampled flock, 15 randomly selected animals were submitted to a double cloacal swabbing. Samples were transported on ice to the National Reference Laboratory for Avian chlamydiosis (NRL, Maisons-Alfort, France). One panel of swabs was stored in conservation buffer SPG [17] at -80°C until inoculated onto chicken eggs. The second panel was stored dry at -80°C until subjected to DNA extraction.

Direct detection of Chlamydiaceae in birds

The dry panel of cloacal swabs was subjected to DNA extraction using the QIAamp DNA Mini Kit, following the buccal swab protocol (Qiagen, Courtaboeuf, France). A *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene was used in this study [15]. All samples with a cycle threshold (Cq) > 38 were considered negative.

Inoculation onto chicken eggs

For cell culture, suspensions of cloacal swabs stored in conservation buffer at -80°C were inoculated onto seven day-old embryonated eggs as previously described [18].

Real-time PCR for detection of *Chlamydia psittaci* and *Chlamydia gallinacea*

All *Chlamydiaceae* PCR-positive samples from humans and animals were re-analysed using previously described real-time PCR assays for *C. psittaci* [16] and *C. gallinacea* [11]. In addition, a new *enoA*-based real-time PCR protocol for *C. gallinacea* was developed in this study. It uses primers *enoA_F* (5'-CAATGGCCTACAATTCCAAGAGT-3'), *enoA_R* (5'-CATGCGTACAGCTTCCGTAAAC-3') and probe *enoA_P* (5'-FAM-ATTCGCCCTACGGGAGCCCCCTT-TAMRA-3') under standard cycling conditions.

DNA microarray and sequencing

A previously described DNA microarray capable of identifying all *Chlamydia* spp. [19] was recently extended to include the new species of *C. avium* and *C. gallinacea* [20]. This array version Chlamydiao4 (Alere Technologies, Jena, Germany) was used throughout the study.

The *ompA* gene was partially amplified from human samples and animal isolates as described previously

using primers CTU/CTL [21] or 191CHOMP/CHOMP371 [22].

Results

Recognition of the outbreak

In May 2013, eight cases of respiratory disease were reported to the public health authorities of Aquitaine, south-western France. As individuals had gutted about a hundred chickens for the preparation of two meals on a poultry farm on the days preceding the onset of clinical signs, they were suspected of psittacosis. The entire group that had participated in these activities on 14 and/or 24 May comprised 15 persons.

A summary of patient information and diagnostic testing is given in Table 1. In four cases, the presence of *C. psittaci* in sputum was demonstrated by real-time PCR. The eight hospitalised cases were treated with antibiotics (macrolides in association with cephalosporins for six days, then only macrolides for seven additional days), and all individuals quickly recovered. Throat swabs collected from four patients after the beginning of their treatment were all negative by PCR.

Finally, four confirmed cases, one probable case and three possible cases were identified. The relatively high DNA content in the samples from Patients 5 and 6 (Cq 28 and 30, respectively) allowed *ompA* sequencing, which revealed identical sequences with 100% homology to *C. psittaci* strain o6-859. This strain was assigned to *ompA* genotype E/B, subtype 859 [5].

Patient characteristics

All patients were hospitalised between 26 and 30 May, with onset of clinical signs recorded between 24 and 28 May (Figure). All were women aged between 42 and 67 years. All except one participated in the preparation of both meals. No previous travel was reported by any of them. All presented fever and two had cough. Headache, vomiting, asthenia, myalgia, dizziness and urinary tract infection was also reported. Type 2 diabetes, rheumatoid arthritis, asthma or cirrhosis were underlying diseases reported for five patients. Unfortunately, due to difficulties in communicating with women from this group, who spoke very little

FIGURE

Psittacosis case distribution by date of disease onset, France, May 2013 ($n = 8$)

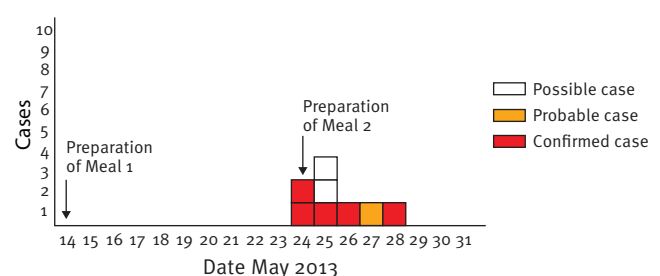


TABLE 2

Diagnostic data and background information on poultry flocks, psittacosis outbreak, France, May 2013 (n = 8)

Id Anses_Flock	Species	Age (days)	Number of animals	Arrival on the farm	PCR <i>Chlamydiaceae</i> CH23S			PCR <i>C. psittaci</i> (<i>incA</i>)		PCR <i>C. gallinacea</i> (<i>enoA</i>)		Isolates	Species previously held on this field		
					Positive/total	Cq values mean (range)	Positive/total	Cq values mean (range)	Positive/total	Cq values mean (range)	Species		Effective	Implementation date	
13-1648-BC1	Chicken	130	4,700	31/01/13	5/15	28.1 (19.2-37.7)	3/5	29.3 (17.7-37.1)	3/5	24.2 (22.4-25.3)	1 <i>C. psittaci</i>	Duck	2,550	08/11/12	
13-1648-BC2	Chicken	67	2,200	05/04/13	15/15	28.6 (24.9-37.7)	3/15	35.7 (34.0-36.7)	14/15	29.3 (25.4-37.5)	6 <i>C. gallinacea</i>	NA	NA	NA	
13-1648-BC3	Chicken	42	2,800	29/04/13	2/15	31.9 (27.1-36.8)	2/2	36.8 (36.5-37.1)	1/2	27.9	No isolate	NA	NA	NA	
13-1648-MD1	Duck	50	2,652	22/04/13	5/15	36.4 (32.9-37.8)	1/5	37.2	3/5	36.0 (33.2-38.4)	ND	Chicken	3,200	28/11/12	
13-1648-MD2	Duck	78	2,652	25/03/13	13/15	34.3 (30.7-37.6)	13/13	33.9 (29.5-37.8)	4/13	37.5 (36.5-37.9)	ND	Duck	2,552	21/12/12	
13-1648-MD3	Duck	34	2,040	07/05/13	12/15	34.8 (28.2-37.8)	4/12	30.2 (26.7-37.9)	7/12	36.8 (36.2-37.8)	ND	Chicken	2,700	02/11/12	
13-1648-MD4	Duck	70	2,941	03/04/13	6/15	35.4 (33.6-36.7)	4/6	33.9 (33.1-34.7)	3/6	37.7 (37.7-37.9)	ND	Duck	2,652	29/01/13	

NA: not available; ND: not done.

French, only the eight patients attending a physician were questioned. Therefore, further epidemiological investigations within the group were not possible.

Examination of samples from poultry

In preliminary testing, swabs from five chickens were examined by real-time PCR, which revealed positivity for *Chlamydiaceae*. These findings prompted a more comprehensive investigation to include all flocks on the site, i.e. three broiler chicken (BC) flocks and four mule duck (MD) flocks. A summary of diagnostic data and information on flocks is given in Table 2. Interestingly, *C. psittaci* was detected in all flocks, as well as the recently introduced species of *C. gallinacea*. In terms of infected animal number and bacterial load, ducks were predominantly infected by *C. psittaci*, whereas chickens were predominantly infected by *C. gallinacea*, except for flock BC1, which also included one high shedder of *C. psittaci* among 15 animals tested. All *Chlamydiaceae*-positive samples were re-analysed with the extended chlamydial microarray that included *C. gallinacea*-specific probes. A very good correlation between real-time PCR and microarray was observed for samples having Cq < 35. No clinical signs were reported in any of these flocks.

Isolates were successfully cultured from BC1 (n=1) and BC2 (n=6) chicken flocks (Table 2). Using real-time PCR, the BC1 isolate was identified as *C. psittaci*, whereas the six BC2 isolates were *C. gallinacea*.

Comparison of human and animal samples

Partial sequencing of the *ompA* gene from the BC1 *C. psittaci* isolate revealed an identical sequence to those obtained from the two PCR-positive patients from whom sequencing was possible. This sequence was also obtained from two duck samples with sufficient DNA content (both from flock MD2). Analysis of the *ompA* sequences from the six *C. gallinacea* isolates of BC2 revealed two distinct groups, which suggests mixed *C. gallinacea* infection in this flock.

Farm management

Frequent rotations between duck and chicken flocks, with flocks sharing the same fields (Table 2), were characteristic for the management of the farm. Interestingly, ducks had previously been raised on the same field on which flock BC1 was established in January.

Discussion

Eight cases of psittacosis (four confirmed and four probable or possible cases) were identified among a group of 15 women who gutted chickens in a confined space on the days that preceded the onset of clinical signs. Initially, infection with Middle East respiratory syndrome coronavirus had been considered because one case had been identified in France in the same month [23]. However, this assumption was discarded in favour of psittacosis as these women suffered from pneumonia or influenza-like symptoms. Clinical signs of psittacosis are similar to those associated with other pathogens that cause pneumonia, so that

clinicians need to include *C. psittaci* in their differential diagnosis, especially when close contact with birds is reported. Knowledge of previous exposure to birds was crucial for the decision on medication of these patients, which included an early and adapted prescription of antibiotics.

In France, *C. psittaci* is widespread in poultry, particularly on duck farms [24], and the most severe human cases reported each year by the NRC are mainly related to ducks, less frequently to pigeons or psittacines. *C. gallinacea* is a newly described chlamydial species [10]. Recent surveys on the prevalence of *C. gallinacea* in poultry flocks in four European countries and China revealed a prevalence that could even exceed that of *C. psittaci* [11], at least for chickens and turkeys. These data were recently confirmed by a survey conducted on *Chlamydiaceae* prevalence in French slaughtered poultry birds, which revealed that *C. gallinacea* is mainly encountered in chickens and turkeys, while *C. psittaci* is most often detected in ducks [25]. On the farm investigated in this study, the same general observation was made, except that *C. psittaci* was also detected in chicken flocks BC1 and BC2, with one animal in BC1 identified as a high shedder (Cq = 17). Sequences of the *ompA* gene from DNA of patient samples and from the *C. psittaci* isolate obtained from BC1 were identical and homologous to the E/B genotype subtype 859. The same *ompA* sequence was obtained from swab samples collected from ducks, suggesting one single *C. psittaci* isolate may have been circulating on this farm and probably represented the origin of the human outbreak. This genotype is commonly identified in *C. psittaci* isolates from French ducks [18]. Interestingly, while chickens and ducks were reared separately on this farm, retrospective analysis of flock rotations showed that ducks had preceded BC1 chickens on the same field. The alternation of poultry species on grasslands probably explains the presence of *C. psittaci* in these chickens alongside *C. gallinacea*. Monitoring faecal shedding could be a way to track the persistence of *Chlamydiaceae* on animals as well as contaminations between flocks.

While *C. gallinacea* has also been detected in the chicken flock harbouring the birds gutted by the patients, DNA extracted from human samples were only positive for *C. psittaci*. The pathogenicity of *C. gallinacea*, a recently discovered species, has yet to be defined [10]. The infectious dose seems to be a critical parameter for an active human infection. In flock BC1, *C. psittaci* was the more prevalent chlamydial agent in terms of bacterial load in infected birds, as very low Cq values were detected. *C. psittaci* antibodies were detected using micro-immunofluorescence testing in only two cases. This is in line with observations from experimental infection of animals, where the humoral immune response to *C. psittaci* infection was generally weak and did not emerge regularly [24]. New serological techniques based on specific oligopeptides are currently under development in order to differentiate

chlamydial antibodies at species level [26]. Such a tool, if extended to include the recently described new species of *Chlamydia*, would be of great value, e.g. to assess the aetiological importance and zoonotic potential of *C. gallinacea*.

Following reports of this psittacosis cluster, the veterinary services made an on-site inspection on the farm and commissioned samples. Slaughtering activities were suspended and farm activities were temporary blocked. Several slaughterhouses were contacted and did not accept to process these poultry birds due to the known risk of psittacosis, so that the animals had to be euthanised on site. This series of events was an opportunity to test the national procedures in place for the emergency management of outbreaks of avian influenza. On the farm, buildings and grasslands were cleaned and/or disinfected and recommendations were given to the farmer on farming practices in order to limit the risk of a new outbreak.

In conclusion, this survey showed that, even if rare in French flocks, chickens can also harbour *C. psittaci*. Farming practices that include grassland rotations of different species should be avoided to prevent the transmission of pathogens from one avian species to another. All individuals involved in activities associated with live poultry birds, especially if done in a confined area, must wear appropriate protective clothing (masks and gloves). It is also important to keep in mind that *C. psittaci* as a zoonotic agent is generally highly prevalent in poultry birds, notably in ducks, despite the absence of clinical signs in carrier animals.

Conflict of interest

None declared.

Authors' contributions

RA, KL, KS wrote the manuscript. IC took part in the clinical management of the patient. RA, BdB, KS collaborated in diagnosis methods. LM, HR, PR, VS, VV collaborated on the public health investigation. FM, JLM collaborated on the veterinary investigations. All authors approved the manuscript.

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