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Chlamydial infections in duck farms associated with human cases of psittacosis in France

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ABSTRACT

Five severe cases of psittacosis in individuals associated with duck farms were notified in France between January and March 2006. Diagnostic examination included serology and/or molecular detection by PCR from respiratory samples.

As a consequence, we investigated all duck flocks ($n = 11$) that were housed in the three farms where human infections occurred. While serology by complement fixation test was negative for all samples, cloacal and/or tracheal chlamydial excretion was detected by PCR in all three units. Notably, one duck flock was tested strongly positive in 2 of the 3 affected farms, and *Chlamydophila* (*C.*) *psittaci* strains were isolated from cloacal and/or tracheal swab samples from both farms.

Human samples and duck isolates exhibited the same PCR-RFLP restriction pattern, which appeared to be an intermediate between genotypes A and B. Analysis of *ompA* gene sequences and comparison to those of the type strains showed that the isolates could not be strictly assigned to any of the generally accepted genotypes of *C. psittaci*. Further analysis by MLVA of the PCR-positive human samples revealed two distinct patterns, which were related to previously isolated *C. psittaci* duck strains.

1. Introduction

Avian chlamydiosis is a well-recognised zoonotic disease caused by the obligate intracellular bacterium *Chlamydophila* (*C.*) *psittaci*, which occurs worldwide.

It was reported that more than 467 avian species could be affected by chlamydial infection (Kaleta and Taday, 2003). The infection is usually systemic and occasionally fatal in birds. The clinical signs vary greatly in severity and depend on the species and age of the bird, as well as the causative strain involved (Andersen, 1997). Avian strains

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Abbreviations: MLVA, multiple locus VNTR analysis; MOMP, major outer membrane protein; VNTR, variable number of tandem repeats; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

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of *C. psittaci* cover at least six serotypes (A to F) as determined by serovar-specific monoclonal antibodies (Andersen, 1991, 1997; Vanrompay et al., 1993). Each of these serotypes was assumed to be associated with a particular category of host species, *i.e.* type A and F with psittacines, type B with pigeons, type C with ducks, type D with turkeys, and type E with pigeons and ratites. More recently, molecular typing methods have been developed, such as restriction fragment length polymorphism of PCR products (PCR-RFLP), which identified genotypes that are adequately reflecting the serotypes (Sayada et al., 1995; Sudler et al., 2004).

As far as the transmission of *C. psittaci* strains from birds to humans is concerned, certain professions like veterinarians, bird breeders, handlers and traders have been reported to face a particular risk (Huminer et al., 1988, 1992; Hinton et al., 1993; Saito et al., 2005). Typical transmission pathways involve inhalation of infectious aerosols during handling of infected animals, carcasses or tissues. Besides, contaminated faeces and feathers may play an essential role in zoonotic transmission. The symptoms of human psittacosis are variable, ranging from severe systemic disease to the complete absence of clinical signs (Andersen and Vanrompay, 2000).

C. psittaci infection in ducks has been reported previously in Europe, Australia and the USA (Chalmers et al., 1985; Arzey et al., 1990; Hinton et al., 1993; Léon et al., 2004; Guérin et al., 2006). Most of the recent human cases seemed to be linked to domestic poultry rather than psittacines (Heddema et al., 2006; Gaede et al., 2008).

In France, five cases of severe pneumonia were observed in February and March 2006. As the infected individuals were in close contact with farmed birds, avian influenza had been initially suspected, but was not confirmed by diagnostic findings. Subsequently, samples from patients and animals present at the three farms affected were examined for chlamydial infection.

In this paper, diagnostic data from real-time PCR, serology and genotyping by PCR-RFLP and multi-locus VNTR analysis (MLVA) are reported to characterise human and animal cases, and the prevailing epidemiological situation is described.

2. Materials and methods

2.1. Humans

2.1.1. Samples

Respiratory samples (throat swabs, tracheal aspirates or bronchoalveolar fluid) from four patients were collected during their hospitalisation, stored at −80 °C and sent to the National Reference Centre (NRC, Bordeaux, France) for chlamydiae in dry ice. Aliquots of early serum from each patient and late serum from two patients were also sent to the NRC (for dates of sampling see Table 1).

2.1.2. Direct detection of *C. psittaci* from human samples

Respiratory samples were analysed for *C. psittaci* using a specific *inc*A real-time PCR protocol (Ménard et al., 2006) and by cell culture in order to obtain isolates. For PCR, clinical samples were extracted by using the automated MagNA Pure DNA extraction (Roche Diagnostics, Meylan, France) (De Martino et al., 2006). The TaqMan real-time PCR was carried out in a final volume of 25 μl containing 5 μl of extracted DNA using an ABI Prism7000 thermocycler (Applied Biosystems, Courtaboeuf, France).

Cell culture was performed on McCoy cells in minimal essential medium supplemented with 1 mM glutamine, cycloheximide, glucose and 5% foetal bovine serum in a biosafety level 3 laboratory. Monolayers were made on 15 mm round coverslip in 1-dram shell vials and inoculated with 500 μl of sample. After 48 h incubation, the monolayers were fixed and stained with monoclonal antibodies recognising genus-specific chlamydial LPS (ImagenTM Chlamydia, Dako, I2L Elitech, Labarthe Inard, France).

2.1.3. Serology

A commercialised immunofluorescence test was used (Chlamydia MIF, Focus, Eurobio, France). This assay can measure responses to IgM and IgG subclasses. Each well

Table 1
Human cases: clinical signs and diagnostic data.

	No case	Sex	Age (years)	First clinical signs	Hospitalisation	Clinical diagnosis	Serology		Respiratory samples	PCR	Culture
							Date	Results			
Plant A	1	M	43	19/02/2006	27/02/2006	Pneumonia	01/03/2006	IgG<1/16, IgM positive	Throat swab sample	Negative	Negative
							03/03/2006	IgG 1/128, IgM positive			
	2	F	46	20/02/2006	26/02/2006	Pneumonia + ARDS	27/02/2006	IgG 1/256, gM positive	Tracheal aspiration	Positive	Positive
Plant B	3	F	51	20/02/2006	27/02/2006	Pneumonia + ARDS	03/03/2006	IgG 1/16, IgM negative	BAL	Positive	Negative
	4	F	44	01/03/2006	04/03/2006	Pneumonia + ARDS	06/03/2006	IgG < 1/16, IgM negative	Tracheal aspiration	Positive	Negative
Plant C	5	M	38	26/12/2005	–	Pneumonia	05/01/2006	IgG 1/64, IgM negative	ND	ND	ND
							23/02/2006	IgG 1/128 IgM negative			

ARDS: acute respiratory distress syndrome; BAL: broncho-alveolar fluid; ND: not done.