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Zoonotic potential of Chlamydophila

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Abstract

The purpose of this article is to present the diseases induced in humans and animals by the different species of *Chlamydophila*, after providing an overview on the history of these infectious agents and their taxonomy. The route of transmission and the available methods for prevention and control in the different animal species are reviewed.

Keywords: *Chlamydophila*, zoonosis, prevention, vaccines

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1. Introduction

Chlamydiaceae are spread globally and cause acute diseases in humans and animals. They may provoke ocular, pulmonary, genital, articular, and intestinal illness, but very often they induce persistent, chronic, or subclinical infections. All *Chlamydophila* species are potential zoonotic pathogens, although *Chlamydophila psittaci* and *Chlamydophila abortus* are the most important and best documented.

Chlamydiae are obligate intracellular gram-negative bacteria with a unique growth cycle, which is initiated by the attachment to the host cell of the infectious elementary bodies (EBs), extracellular dormant, particles with a cell wall quite similar to that of Gram negative bacteria. Adhesion induces phagocytosis within a phagosome. After entering the cell, the EBs convert into vegetative reticulate bodies (RBs), in which macromolecular synthesis takes place. After multiplication by binary fission, the RBs convert back into EBs, which are released from the host cell by lysis or exocytosis.

2. History

In the history of chlamydial infections, the description of trachoma first occurred in the Ebers papyrus (1500 B.C.) and in ancient Chinese manuscripts. Pedanius Dioscorides, a Sicilian physician, first used the term trachoma in 60 A.D. A century later the four stages of the disease were delineated by Galen (Thygeson, 1962).

At the beginning of the 19th century, a relationship was suspected between an infection in psittacine birds and pneumonia in humans who had been in contact with them. In 1895, Morange proposed the name psittacosis for this infection, but the disease-causing agent was finally isolated 35 years later during an epidemic of several hundred cases caused by infected

Argentinean parrots in 1929-1930 in the US and Europe. In 1930, Levinthal described minuscule spherical basophilic bodies in tissues of infected parrots, and almost simultaneously, Coles and Lillie made the same observation in the cells of infected humans and birds. Bedson and co-workers (Bedson et al., 1930) established the etiological role of these organisms in the disease. In 1932, the intracellular developmental cycle of the psittacosis agent was described. Two years later, Thygeson (Thygerson, 1934) identified a resemblance between the developmental cycle of the psittacosis agent and the organisms observed during the replication of the trachoma and inclusion conjunctivitis agents, which were first found in 1907 by Halberstaedter and von Prowazek (Halberstädter and von Prowazek, 1907). EBs were detected in the brains of cattle suffering from sporadic encephalomyelitis (McNutt and Waller, 1940), in the lungs of asymptomatic mice (Gönnert, 1941), and in cats suffering from pneumonia (Baker, 1944). In 1950, Stamp and co-workers (Stamp et al., 1950) demonstrated that *Chlamydiae* caused abortion in sheep, and more recently, *C. psittaci* strains were isolated from tortoises and *C. pneumoniae* from frogs and snakes (Bodetti et al., 2002).

However the nature of these pathogenic agents was only clarified at the end of the 1960s. They were originally regarded as viruses because they were unable to grow outside of a live cell, then as an intermediate organism between bacteria and viruses (Bernkopf et al., 1962) because clear definitions of bacteria and viruses were lacking. The cultivation of the psittacosis agent in the chorioallantoic membrane (Burnet and Rountree, 1935), then in the yolk sac of chicken embryos (Yamura and Meyer, 1941), allowed biochemical and biological studies showing that these agents are prokaryotic organisms that parasitize eukaryotic cells. They retain their cellular properties throughout the growth cycle, and the RBs develop in cytoplasmic vacuoles and divide by a process analogous to binary fission in bacteria. Both the EBs and RBs contain DNA and RNA, and the cytoplasm is defined by a cell wall. They are

sensitive to antibiotics, leading to the conclusion that they share many properties with bacteria (Moulder, 1966).

3. Taxonomy

The classification was controversial until recently, as attested to by the various appellations that designated the agents of the PLT group, Psittacosis, Lymphogranuloma venereum Trachoma, over the years. In 1948, they were classed in the order *Rickettsiales* and the family of *Chlamydozoceae* Moshkowsky, which included three genera (*Chlamydozoon*, *Myagawanella*, and *Colesiota*), since they could not be cultivated outside of living animal cells. During the next 20 years, a new classification was proposed almost every year until Storz and Page (Storz and Page, 1971) proposed placing them in a new order, *Chlamydiales*, which contained only one genus, *Chlamydia*, characterized by the unique intracellular developmental cycle. This genus included two species, *Chlamydia trachomatis* and *Chlamydia psittaci* (Page, 1968) that are distinguished on the basis of sulfonamide susceptibility and glycogen accumulation within the inclusion, leading to different iodine staining. *C. psittaci* strains were sulfadiazine resistant and iodine-staining negative, whereas *C. trachomatis* strains were sensitive to sulfonamides and iodine-staining positive. In addition, the two species appeared to have a low degree of similarity in DNA sequences (Weiss et al., 1970).

Molecular biology and phylogenetic analysis of the 16S and 23S rRNA genes changed the classification of *Chlamydia* and the nomenclature of the strains again (Everett et al., 1999). Now the order *Chlamydiales* contains at least four families and the family *Chlamydiaceae* contains two genera, *Chlamydia* and *Chlamydophila*, with nine species: three for the genus *Chlamydia*, *Chlamydia trachomatis*, *Chlamydia muridarum*, and *Chlamydia suis*; two for the genus *Chlamydophila*, *Chlamydophila pneumoniae* and *Chlamydophila pecorum*; and four species that corresponded to the previous *Chlamydia psittaci*,

Chlamydophila psittaci pathogen for birds, *Chlamydophila abortus* pathogen for ruminants, *Chlamydophila caviae* pathogen for guinea pigs, and *Chlamydophila felis* pathogen for cats.

The phylogenetic trees constructed from five other genes, *ompA* coding for the major outer membrane protein (MOMP), *GroEL* coding for a chaperon protein, *omp2* coding for the 60 kDa cysteine-rich protein, *omp3* coding for a small cysteine rich lipoprotein, and *kdtA* coding for the KDO-transferase, supported this new classification (Bush and Everett, 2001). In addition, this classification, which is relevant to the host range, disease syndrome, and virulence, provides easy tools for identifying new strains by PCR-RFLP on the 16S-23S spacer region (Everett et al., 1999) or by DNA microarray (Sachse et al., 2005). Genetic studies suggested that *C. pneumoniae* and *C. pecorum* are closely related and that *C. abortus* is derived from *C. psittaci* (Van Loock et al., 2003).

The revised taxonomy has been adopted by most researchers working on animal chlamydiosis but is still rejected by some involved in human infections (Schachter et al., 2001) that contest the sharing in two genera (Stephens, 2008). In the current literature, these bacteria are designed as *Chlamydia* or *Chlamydophila*, which are abbreviated as *C* and *Cp*.

C. abortus strains exhibit very little antigenic (Salinas et al., 1995) or molecular diversity (Laroucau et al., 2009), but *C. psittaci* could be classified in six serovars (A to F) using anti-MOMP monoclonal antibodies (mAbs) (Andersen, 1997). These serovars are preferentially associated with some host species: A with psittacine birds, B with pigeons, C with ducks and geese, D with turkeys, E with pigeons and ducks, and F with parakeets. The serovars correlate with the genotypes defined by *ompA* polymorphisms characterized by restriction fragment length polymorphisms (RFLP) or sequencing that allowed the identification of type E/B in ducks (Geens et al., 2005).

4. Clinical signs

Table 1 gives an overview of the clinical signs induced in animals and humans by the different species.

Chlamydophila psittaci

Psittacosis is the animal chlamydiosis that presents the major zoonotic threat. *C. psittaci* strains induce a systemic disease in psittacine birds, domestic poultry, and wild fowl. People in frequent contact with domestic and companion birds at work or in their spare time are the most predisposed to infection. The incubation period typically lasts 1 to 2 weeks, but longer incubation periods are also reported. The symptoms are variable and unspecific, resembling mild flu-like illness with fever, headache, sore throat, myalgia, and diarrhea, but endocarditis, encephalitis, and severe interstitial pneumonia with non-productive cough, sometimes fatal, are also reported (Petrovay and Balla, 2008). In addition, the chlamydial infection is often complicated by secondary bacterial infections. With *C. psittaci* strains, delays before antibiotic treatment and the age and immune status of the patient affect disease development, which is more severe in elderly, young, and immunocompromised individuals and pregnant women. Indeed, abortion after contamination by infected birds occurred in pregnant women suffering from pneumonia and respiratory distress (Idu et al., 1998; Khatib et al., 1995). However, the disease is rarely fatal in humans when it is diagnosed rapidly and treated accurately. Thus, awareness of the danger is important for those at risk.

Disease severity in birds depends on the virulence of chlamydial strains and the species of the bird. These factors are not independent because the various genotypes that impact host specificity also show different virulence levels. Nevertheless, asymptomatic infections can occur with strains of both low and high virulence, and many strains remain quiescent in birds until activated by stress. In addition, age can affect disease course: adult birds may have asymptomatic infections, while young birds have acute disease (Herrmann et al., 2006).

Infected psittacine birds often present with hyperthermia, anorexia, lethargy, fluffed feathers, closed eyes, and diarrhea with green watery droppings. Birds may also present with respiratory signs, such as nasal discharge, sneezing, and open-mouth breathing, due to pneumonia and airsacculitis. Conjunctivitis, with ocular discharge, together with sinusitis may be observed. The liver is usually affected and unusual feather coloration from altered metabolism may occur in chronically infected birds (Billington, 2005).

In turkeys, serovar D strains induce the most severe disease, with conjunctivitis, rhinitis, sinusitis, tracheitis, airsacculitis, pneumonia, pericarditis, enteritis, and reduced egg production. Mortality rates may be high without early antibiotic treatment. In addition, serovar D strains are especially dangerous for poultry workers, particularly in the poultry processing plant. Clinical signs of turkeys infected with serotypes of low virulence are milder, including anorexia and diarrhea (Vanrompay et al., 1995b).

In contrast, infected feral pigeons are asymptomatic latent carriers of *C. psittaci* that shed bacteria in feces, respiratory, and conjunctival secretions (Magnino et al., 2008). However, clinical signs, including anorexia, diarrhea, and respiratory signs, may occur in cases of simultaneous disease, such as salmonellosis, trichomoniasis, and viral infections (herpesvirus or paramyxovirus) (Longbottom and Coulter, 2003). Infections in ducks are also often asymptomatic, but confinement and close contact with them may induce severe pneumonia in humans (Laroucau et al., 2008). In some cases, *C. psittaci* strains may infect other mammals, such as dogs (Sprague et al., 2008), horses (Theegarten et al., 2008), and pigs (Kauffold et al., 2006), and induce abortions or pneumonia.

4.2 *Chlamydophila abortus*

C. abortus is the causal agent of enzootic abortion of ewes (EAE) or ovine enzootic abortion (OEA) and is the most common infectious cause of abortion in many small ruminant-rearing countries. The economic impact of these abortions is very high since one third of the pregnant

ewes in a flock and occasionally 2-fold more pregnant goats may abort following the first exposure to *C. abortus*. This high level of abortion persists during 2 or 3 years until almost all the females abort. Then the disease takes on a cyclic nature and only 1% to 5% of abortions occur for several years until a new outbreak, when all primiparous females abort. This cyclic evolution is due to life-long immunity following abortion that protects against subsequent infection (Rodolakis et al., 1998) It is exceptional for a female to abort twice. Cattle, pigs, deer, and horses may also be infected with *C. abortus*.

In addition to the economic losses, *C. abortus* presents a zoonotic risk, inducing mild influenza-like illness or pneumonia in rare cases. Human infections associated with bovine chlamydial pneumonia and bovine encephalomyelitis were recorded (Johnson, 1983; Stepanek et al., 1983), and serological changes and urogenital symptoms were described in men and women in contact with bovine chlamydiosis (Stepanek et al., 1983). Although *C. abortus* is not very contagious in humans, the consequences of the infection for pregnant women in close contact with infected sheep and goats are disastrous. The association of human abortions with *Chlamydia* from ruminants was suspected long ago (Giroud et al., 1956) by several authors (Beer et al., 1982; Crosse et al., 1971; Roberts et al., 1967; Tersikh et al., 1977), but it was based only on serological data. The role of *C. abortus* was contested (Hobson and Rees, 1982) until the isolation of the bacteria from the placenta and the fetus of a young woman who had assisted with a lambing on her husband's farm, on which some ewes had aborted (Buxton, 1986; Wong et al., 1985). RFLP characterization of the two strains from human and ovine origin proved definitively that the young woman was contaminated by her ewes (Herring et al., 1987). Abortions due to *C. abortus* have been confirmed in several other countries, including France (Villemonteix et al., 1990), the US (Jorgensen, 1997), Switzerland (Pospischil et al., 2002), Netherlands ((Kampinga et al., 2000), and Italy (Walder et al., 2005). A fever with headache, malaise, nausea, and vomiting are generally the first symptoms of the

infection in pregnant women and are frequently associated with lower abdominal pain followed by abortion and sometimes severe complications, such as acute renal failure, disseminated intravascular coagulation, or respiratory distress necessitating mechanical ventilation. Recovery from the disease follows termination of the pregnancy and appropriate antibiotic therapy, except in one case when the patient died (Beer et al., 1982).

C. abortus infection of pregnant ewes and goats induces abortion, stillbirth, or delivery of weak lambs or kids that quickly fall ill or have difficulties rising. Abortions mainly occur in the last month of gestation without clinical signs until abortion is imminent. Sometimes vaginal discharge is observed a few days before abortion, more frequently in goats than in ewes. As in small ruminants, no clinical symptoms are observed before the onset of bovine chlamydial abortion, which usually occurs during the last trimester of gestation, but may also take place as early as the fourth month (Storz, 1971).

Usually, aborting females recover rapidly without ill effect. Placental retention is rare in ewes but is more frequent in both cows and goats. This may lead to metritis, which is fatal in a very small number of cases. Arthritis, conjunctivitis, and pneumonia may affect calves, kids, and lambs born live from infected females. The fetus does not show specific macroscopic lesions.

In non-pregnant females, the infection generally develops towards an asymptomatic form, which may induce abortion during later gestations only in a small percentage of animals (Waldhalm et al., 1971). Male fetuses can be contaminated *in utero* (Rodolakis and Bernard, 1977) and adult males by mating with infected females. The infection can reach the seminal vesicles, causing epididymitis, and testicles, affecting the semen quality with shedding of the bacteria in the sperm.

In pigs, *C. abortus* may be isolated from cervical swabs and genital tracts of sows with repeated return to oestrus, abortions, and small litters with weak piglets (Thoma et al., 1997,

Hoelzle et al., 2000, Camenisch et al., 2004;). However, *C. suis* and *C. pecorum* seem to dominate in porcine abortions.

4.3 *Chlamydophila felis*

In cats, *Chlamydophila felis* causes conjunctivitis associated with severe swelling of the lid, mild rhinitis, ocular and nasal discharges, fever, and lameness, as shown by experimental infections (Masubuchi et al., 2002; TerWee et al., 1998) In addition. *C. felis* may reach the genital tract, producing chronic salpingitis leading to persistent oviduct infections.

Due to the close contact between cats and their owners, *C. felis* was suspected of causing human disease, particularly follicular conjunctivitis (Ostler et al., 1969; Schachter et al., 1969) or atypical pneumonitis (Cotton and Partridge, 1998). Endocarditis with secondary glomerulonephritis (Regan et al., 1979) and severe liver breakdown in an immunocompromised patient (Griffiths et al., 1978), both probably caused by a feline conjunctivitis agent, were reported based on serological investigations. However, *C. felis* was only isolated from conjunctivitis. The *Chlamydophila* recovered from the HIV positive patient's eye suffering of chronic conjunctivitis was indistinguishable of that isolated from the cat's eye (Hartley et al., 2001). *C. felis* can induce conjunctivitis in people who are in close contact with infected cats, but the risk is extremely low, as indicated by the comparison of the seroprevalence in cats and their owners (Yan et al., 2000). However, some precaution is warranted when handling affected cats.

4. 4 *Chlamydophila caviae*

C. caviae in guinea pigs produces asymptomatic infection or clinical signs ranging from mild to severe conjunctivitis, with profuse purulent ocular discharge sealing the eyelids. Conjunctival chemosis, follicular hypertrophy, and pannus may develop as early as five days after experimental infection. Keratoconjunctivitis is self-limiting and clears up within three to

four weeks. It may also cause infection of the genital tract, inducing symptoms comparable to those caused by *C. trachomatis* in humans. Infection of guinea pigs with *C. caviae* is widely used as an experimental model of *C. trachomatis* genital tract infection in humans (Batteiger and Rank, 1987).

PCR analysis allowed the detection of *C. caviae* in a conjunctival swab of the owners of symptomatic guinea pigs (Lutz-Wohlgroth et al., 2006). He reported mild serous ocular discharge, suggesting a zoonotic potential for *C. caviae* similar to that of *C. felis*. Symptomatic and asymptomatic guinea pigs may harbor *C. caviae*. As some are pets in close contact with young children, it would be interesting to investigate more accurately if *C. caviae* could be responsible for conjunctivitis in humans.

4.5 *Chlamydophila pneumoniae*

C. pneumoniae, as all *Chlamydophila* species, induces asymptomatic and chronic infections. It was first considered an exclusively human pathogen responsible for respiratory diseases, such as pneumonia, chronic bronchitis, and asthma, but also chronic diseases, such as atherosclerosis. However, strains of *C. pneumoniae* are widespread in the environment. They have been found, by molecular methods in a range of reptiles (snakes, iguanas, chameleons, turtles) and amphibians (frogs), and also in the respiratory tracts of horses, koalas (Bodetti et al., 2002), and western barred bandicoots (Kutlin et al., 2007). *C. pneumoniae* DNA may be found in ocular and urogenital samples of asymptomatic or symptomatic koalas, but in the latter case it is associated with *C. pecorum*, suggesting that *C. pecorum* is responsible for the clinical signs (Jackson et al., 1999). Three biovars have been described (Everett, 2000): the human biovar, also designated TWAR, the equine biovar, and the koala biovar. The genotyping of *C. pneumoniae* DNA from human and non-human origin revealed that most of the strains infecting humans were similar, unlike those from animals. The koala genotype was identified in the carotid plaque of one human (Cochrane et al., 2005)

and strains very similar, if not identical to the human respiratory strains, were found in reptiles and amphibians (Bodetti et al., 2002). Although the zoonotic potential of *C. pneumoniae* remains unclear, *C. pneumoniae* is now so prevalent in human that it is unknown if animals or humans were the “original” reservoir.

4.6 *Chlamydophila pecorum*

Only three *Chlamydophila* species, *C. pecorum*, *C. pneumoniae*, and *C. psittaci*, may be isolated from the brain tissue of infected hosts; however most *C. pecorum* and *C. pneumoniae* strains are not considered highly virulent. Indeed, most healthy ruminants harbor *C. pecorum* in their intestinal tract (Jee et al., 2004) despite the fact that, in addition to encephalomyelitis, this bacterium may induce several clinical signs, such as pneumonia, arthritis, conjunctivitis, enteritis, urinary tract disease, metritis, and fertility disorders, in small ruminants, cattle, swine, and koalas (Table 2). For koalas, *C. pecorum* seems more pathogenic than *C. pneumoniae*.

This species presents many antigenic and genomic variations, particularly on the MOMP (Denamur et al., 1991; Fukushi and Hirai, 1992; Kaltenboeck et al., 2008; Kuroda-Kitagawa et al., 1993; Salinas et al., 1995). Pathogenic (isolates from clinical samples) and non-pathogenic (isolates from the intestinal tracts of healthy ruminants) strains differ genetically (Yousef Mohamad et al., 2008a). Phylogenetic analysis could distinguish them based on the sequence of three potential virulence genes, *ompA*, *incA*, which encodes an inclusion membrane protein, and an ORF including a 15-nucleotide variant coding for a tandem repeat (Yousef Mohamad et al., 2008b). However, its potential zoonotic risk is still unknown.

5. Route of transmission.

Chlamydophila is shed in feces, urine, respiratory secretions, birth fluids, and placentas of infected symptomatic and asymptomatic animals, and in saliva and feather dust

of birds. As EBs do not survive very long, close contact is needed with infected animals to become infected. Two factors affect the transmission of the disease: the virulence of the strains and the number of bacteria that infect the host. The minimal infecting dose of the different strains are still unknown, but some serovars of *C. psittaci* are considered very contagious for humans, unlike *C. abortus*, but the quantity of bacteria shed in the placenta of an aborting ewe or goat is much higher than that shed by birds.

Transmission of *C. psittaci* and *C. abortus* from animals to humans generally happens through inhalation of infectious dust and aerosols inducing infection of the mucosal epithelial cells and macrophages of the respiratory tract (Table 1). The bacteria eventually spread through the blood stream to involve epithelial cells and macrophages of various organs, including the placentas of pregnant women. *C. psittaci*, *C. abortus*, and *C. pneumoniae* may be detected in the PBMCs (peripheral blood mononuclear cells) of infected humans or animals, whereas *C. felis*, *C. pecorum*, and *C. caviae* tend to cause local infection of the mucosa. The rate of the clearance of *C. abortus* in the blood depends on the initial inoculum. *C. felis* may be transmitted from infected cats to humans via the fingers of the owners contaminated by the ocular discharges or infected dust in the fur of the pet.

In addition to the respiratory and ocular routes, the oral route is also important for the transmission of *C. psittaci* and *C. abortus* in animals. Drinking infected water could be a source of contamination for many avian species, while predators and scavengers may become infected by ingestion of infected carcasses and water. In addition, transmission from the parent to young birds may occur at the time of feeding by regurgitation (Harkinezhad et al., 2008).

In ruminants, the infection often originates from the ingestion of placenta or grass contaminated by vaginal discharge and placenta. Goats and ewes are more liable to abort when they are infected within 100 days of gestation. However, in intensive lambing

conditions, contamination at the time of lambing can lead to abortion during the following gestation (Wilsmore et al., 1984).

Vertical transmission, which promotes the persistence of the infection in ruminant herds, also occurs in birds. The infection of eggs leads to embryonic death or to the hatching of live infected young birds. A major route of infection for neonatal kittens is from the mucosal surface of the reproductive tract of their mother at birth.

The venereal transmission of *C. abortus* is possible as the bacteria are found in the semen of bulls, rams, goats, and boars. Males become infected *in utero* or after mating of infected females. However, during post-abortion ovulation, no or very few DNA copies may be detected by PCR (Livingstone et al., 2008). In addition, venereal transmission to females leads to sterility and early embryonic mortality rather than late abortions.

In the nest, *C. psittaci* may be transmitted by lice, mites, and flies (Longbottom and Coulter, 2003). A similar role for other arthropod vectors has never been demonstrated for other *Chlamydophila* species. Birds appear to be excellent vectors for the distribution of chlamydial infection because they feed on and have access to the feces and carcass of infected animals and are highly mobile (Everett et al., 1999). Although the isolation *C. abortus* from birds is extremely rare (Herrmann et al., 2000), it has been isolated from cats and dogs on farms with infected ruminants (Salinas et al., 1995).

6. Diagnostics

Definitive diagnosis is obtained by identification of the organism. This can be achieved by microscopic examination of stained smears, by detection of Chlamydial antigen by immunofluorescence or by ELISA, whereas Chlamydial DNA can be detected by the polymerase chain reaction (PCR) or by microarray. Several commercial PCR kits are available.

The possibilities for diagnostic detection of chlamydiae improved considerably following the introduction of PCR, which permits direct identification from clinical specimens and differentiation of species. The methodologies and technologies used currently in diagnosis of chlamydial infections in animals have recently been reviewed (Sachse et al., 2008b).

The determination of the *Chlamydophila* species and the genotype for *C. psittaci* by PCR and microarray (Sachse et al., 2008a) is important because the species do not present the same zoonotic risk.

Serological analyses can help confirm the molecular diagnosis or in epidemiological studies or if the identification of the agent is not feasible. The standard serological test for chlamydial antibodies is the complement fixation test (CF) using a group-reactive lipopolysaccharide antigen present in all strains. The occurrence of high CF titer in the majority of individual in a flock is presumptive evidence of active infection. The demonstration of a four fold increase in titer in an individual bird is considered to be diagnostic of a current infection. In ruminants, CF test lacks specificity due to its antigen common to *C. pecorum*. Several ELISA tests *C. abortus*-specific have been developed in order to improve chlamydial serology (Vretou et al., 2007). However, whatever the serological test it cannot identify carriers and potential shedders

In human infection is generally diagnosed by seroconversion on paired acute and convalescent phase sera, although a single acute phase titer in the setting of clinically compatible illness is significant. Low positive titers are common in people in contact with animals. False positives may occur in *C. pneumoniae* and *C. trachomatis* infections. Specific micro-immunofluorescent (MIF) test must be used to improve the diagnosis. However a recent study demonstrated the weakness of MIF test for diagnosing human psittacosis (Verminnen et al., 2008).

7. Antibiotic Therapy

Tetracycline is generally used for the treatment of animal chlamydiosis. *Chlamydiae* are sensitive to several antibiotics, such as cyclines, quinolones, and macrolides. However, these antibiotics are not bactericidal and the therapeutic levels of the drug should remain for a long time (several weeks) to eliminate the infection and avoid the risk of transmission to humans. These long-term treatments are generally too expensive and not used for ruminants.

Most human patients respond to orally administered doxycycline or tetracycline hydrochloride, however the treatment must continue for at least 10 to 14 days after fever abates (<http://www.nasphv.org/Documents/Psittacosis.pdf>).

7.1 *Chlamydophila psittaci*

As there are no vaccines available, antibiotic treatment usually with tetracycline is the only way to limit the infection. Treatment needs to be maintained for extended periods of time. For pet birds, 45 days is often recommended (Vanrompay et al., 1995a). Antibiotics may be given with food (chlortetracycline 500-5000 ppm according to the bird species and the type of food, doxycycline 1000 mg/kg, or enrofloxacin 250-1000 ppm) (Gerlach, 1999) or in water (doxycycline 200-800 mg/L according to the bird species) but it is necessary to verify that no other source of water is available for drinking. Better results are obtained with doxycycline in water, since it is more stable in water than food and generally more accepted by birds, except for psittacine birds, which do not drink enough. In addition, parrots may be very suspicious of anything added to their water (Billington, 2005). For individual pet birds, direct treatment allows better control of the level of antibiotic in the blood. Intramuscular injections of doxycycline provide the best results. After antibiotic treatments, bacterial clearance should be checked at the end of the treatment and periodic re-sampling is advisable to monitor relapse.

Treatments must be used only to cure sick birds. Preventive regular use of antibiotics should be avoided to prevent antibiotic resistance. Although no resistance has been found in *C. psittaci* strains, tetracycline-resistant *Chlamydia suis* have been isolated from pigs in several countries (Di Francesco et al., 2008; Dugan et al., 2004).

7.2 *Chlamydophila abortus*

In ruminants, antibiotic treatment consists of two injections of tetracycline (20 mg/kg) during the last month of gestation to reduce the number of abortions and the quantity of *C. abortus* shed at parturition (Rodolakis et al., 1980). However, the infection is not cured and the shedding of the bacteria persists as well as the threat for pregnant women. This kind of treatment must be used only to prevent a high rate of abortion at the time of the first disease outbreak in the flock, and then the animals must be vaccinated.

7.3 *Chlamydophila felis*

Doxycycline is generally recommended for the treatment of conjunctivitis in cats. Oral treatment with 10 mg/kg is more efficacious than antibiotic eye ointment, but several weeks of treatment (at least 4 weeks) are needed to avoid relapse (Dean et al., 2005). Infected cats should be kept indoors to prevent the spread of the infection. In the case of outbreaks in a cattery, all cats must be treated and infected cats must be separated from the others to minimize exposure.

8. Vaccines

8.1 *Chlamydophila psittaci*

No commercial vaccine is available for avian chlamydiosis despite work on vaccines for turkeys and, the good protection obtained in turkeys by plasmid DNA vaccines expressing the MOMP of *C. psittaci* serovar A (Vanrompay et al., 2001; Vanrompay et al., 1999), and

the demonstration of the MOMP expression for at least ten weeks after vaccination with such vaccine (Loots et al., 2006).

8.2 *Chlamydophila abortus*

Both live and inactivated vaccines for *C. abortus* have been developed. The live thermosensitive vaccine (Ovilis Enzoowax® or Cevac® Chlamydia) is more efficient and induces the same immunity as the natural disease (Rocchi et al., 2008), which reduces the clinical signs and prevents infection in challenged ewes and goats (Rodolakis and Souriau, 1983, 1986). This live vaccine may be combined with other live vaccines against brucellosis, salmonellosis (Souriau et al., 1988), and toxoplasmosis (Chalmers et al., 1997). It could be compatible with the Q fever phase 1 vaccine (Coxevac CEVA) at two different injection sites as demonstrated in mice (Rekiki et al., 2004b). It is effective against all *C. abortus* strains tested and *C. pecorum* (Rekiki et al., 2004a), but not against *C. psittaci*. However, vaccination induces the production of antibodies that cannot be differentiated from those of naturally infected animals (Borel et al., 2005). Until now, no subunit or recombinant vaccines are as effective as the live vaccine (Longbottom and Livingstone, 2006). In contrast to *C. psittaci*, plasmid DNA vaccines expressing the MOMP of *C. abortus* either did not protect mice against a virulent challenge (Hechard et al., 2003) or less effectively than the live vaccine (Zhang et al., 2008).

No boosters are needed for about five years, but young animals and those newly introduced to the flock must be vaccinated during those five years. Indeed, abortion may persist during three to five years after the vaccination due to the infected females that give birth to infected young susceptible to abortions. The live vaccine is very safe. Thousands of ewes and goats have been vaccinated without problems, but pregnant females should not be vaccinated.

8.3 *Chlamydophila felis*

Both live and inactivated vaccines for *C. felis* have been used in Europe, the US, and Japan. The live attenuated vaccine is more efficient and reduces clinical signs, but does not completely prevent infection (Shewen et al., 1980). This live vaccine may be combined with a trivalent live vaccine against herpesvirus, calicivirus, and panleukopenia virus (Starr, 1993). In addition, a new specific antigen should distinguish between infected and vaccinated cats, as high levels of antibodies against this antigen are detected only in the sera of infected cats (Ohya et al., 2008). Vaccination of cats living in close contact (cattery, pet shops, or pet keepers) is recommended.

9. Conclusion

The prevalence of chlamydial infections in humans, including the psittacosis, is likely underestimated in many countries, including the US, Australia, and several European countries, such as Belgium, Germany, and Switzerland, where it is a notifiable disease.

A cheap and efficacious vaccine against *C. psittaci* is needed to prevent infection in poultry and avoid contamination of workers in turkey and duck processing plants. Genetic vaccines are too expensive and probably not acceptable to consumers but could be considered for pets birds to reduce the use of systematic antibiotic treatments, or perhaps for at risk humans.

Everyone in contact with birds should be informed about the clinical signs of chlamydial infection and quickly seek medical attention for themselves and their pets if they have any suspicion of contamination. In addition, routine hygienic precautions are recommended when handling and feeding birds and cats. Children must be advised to avoid close face-to-face contact with cats and guinea pigs with conjunctivitis. Pregnant women, especially those who live in rural areas, should be made aware of the risks of zoonotic diseases and how to avoid them.

Conflict of Interest Statement

The authors certify that they have no financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Zoonotic Potential of *Chlamydophila*”.

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Table 2 Clinical signs induced by *C. pecorum* according to host species

Clinical signs	Ewes and goats	Cattle	Swine	Koalas
encephalomyelitis	-	+	-	-
pneumonia	+	+	+	+

arthritis	+	+	+	-
conjunctivitis	+	+	+	+
abortion	<u>±</u>	<u>±</u>	+	-
metritis and infertility	-	+	+	+
enteritis	-	+	+	+
urinary tract disease	-	-	+	+

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Table 1 Zoonotic potential of the *Chlamydophila* species and routes of transmission from animals to humans

<i>Chlamydophila</i> species	Host		Pathology in animals		Route for human transmission	Pathology in humans	
	Principal host	Occasional host	Clinical signs	Severe disease		Usual disease	Severe disease
<i>C psittaci</i>	Bird	Dog, horse, pig	Hyperthermia, anorexia, lethargy, diarrhoea	Conjunctivitis pneumonia, pericarditis, death	Inhalation	Influenza-like illness	Endocarditis, encephalitis, pneumonia, death
<i>C abortus</i>	Sheep, goat cattle	Pigs, deer, horse	Abortion, stillbirth, epididymitis ¹	Metritis	Inhalation	Influenza-like illness	Pneumonia, abortion, renal failure, respiratory distress, death
<i>C felis</i>	Cat	- ²	Conjunctivitis	Pneumonia, chronic salpingitis	Contact	Conjunctivitis	Endocarditis, severe liver breakdown
<i>C caviae</i>	Guinea-pig	-	Conjunctivitis, genital tract infection	-	Contact	Conjunctivitis	-
<i>C pneumoniae</i>	Human koala, horse	Reptiles, amphibians	Respiratory diseases	-	Inhalation	Pneumonia, bronchitis, asthma ³	Atherosclerosis ³

<i>C pecorum</i>	Ruminant, swine, koala	Wild animal	Intestinal				
			infection,	Encephalomyelitis,			
			conjunctivitis,	pneumonia,	Contact	-	-
			urinary tract	arthritis			
			disease				

- 1- clinical sign in infected male
- 2- (-) Not reported
- 3- Clinical signs by human *C pneumonia* isolates