Chapter 24
Avian Chlamydiosis

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Introduction

Definition and Synonyms

Avian chlamydiosis (AC) is defined as an infection with a *Chlamydia* species in birds. The taxonomy of the family *Chlamydiaceae* was recently revisited. The genus *Chlamydia* includes currently 11 recognized species, and among them *C. psittaci*, *C. avium*, *C. gallinacea* and occasionally *C. abortus* have been isolated from birds (34, 75, 81, 89).

Avian chlamydiosis is a respiratory disease, usually systemic and occasionally fatal. This chapter primarily covers current knowledge on *Chlamydia psittaci* infections as they occur in birds raised commercially for meat and egg production. It should be noted that the disease in pet birds is quite similar, and the disease characteristics, transmission, and diagnosis are essentially the same.

Chlamydia psittaci can be transmitted to humans. The disease in birds and humans originally was called psittacosis or parrot fever because it was first recognized in psittacine birds and in humans associated with psittacine birds. Today we speak of chlamydiosis in birds and psittacosis in humans.

Public Health Significance

The strains of avian chlamydiae can infect humans and should be handled with appropriate biosafety and containment procedures. Risk assessment and management are essential when performing diagnosis of AC. Adequate information, communication, and health surveillance by an occupational physician are recommended (19, 82).

Most infections occur through inhalation of infectious aerosols. While the disease from psittacine birds is best known, the infection in poultry is of particular concern as transmission to humans is common during handling and slaughter of the birds (20, 21, 42, 46, 48, 49). Post-mortem examinations of infected birds and handling of cultures should be done in certified Class II laminar flow hoods whenever possible or with proper protective equipment. Appropriate zoonotic agent decontamination procedures should be followed because human infection can result from transient exposures. The incubation period is usually 5–14 days; however, longer incubation periods are known. Human infections vary from inapparent to severe systemic disease with interstitial pneumonia and encephalitis. The disease is rarely fatal in properly treated patients; therefore, awareness of the danger and early diagnosis are important. Infected humans typically develop headache, chills, malaise and myalgia, with or without signs of respiratory involvement. Pulmonary involvement is common. Auscultatory findings, however, may appear to be normal or to underestimate the extent of involvement. Diagnosis can be difficult and in the past was usually established through testing paired sera for antibodies to chlamydia by the complement fixation (CF) test. However, some patients remain

seronegative although being hospitalized due to psittacosis. Thus, serology is more and more being replaced by nucleic acid amplification techniques (NAAT's), also because they allow bird source tracing. In humans, tetracycline, doxycycline, or azithromycin are usually the drugs of choice unless contraindicated. The length of treatment will vary with the drug, but should be continued for at least 14 days for tetracycline. Secondary spread among humans rarely occurs (124). Because the disease is rarely fatal in properly treated patients, awareness of the danger and early diagnosis are important.

History

The first description of a psittacosis outbreak dates from 1879 and was described by Jacob Ritter, linking the disease to pet parrots and finches (72). Historical aspects of chlamydia-related diseases in animals and humans are reviewed by Pospischil (69).

Etiology

Classification

The members of the family *Chlamydiaceae*, order *Chlamydiales* are obligate, intracellular Gram-negative bacteria. In 1999, Everett et al. (25) proposed a reassignment from the single genus *Chlamydia* into 2 genera, *Chlamydia* and *Chlamydophila*, based on clustering analyses of the 16S rRNA and 23S rRNA genes (Table 24.1). However, recent comparative genome analyses are consistent with the conclusion that host-divergent strains of chlamydia are biologically and ecologically closely related (45, 85). The previous taxonomic separation of the genus based on ribosomal sequences is neither consistent with the natural history of the organism revealed by genome comparisons. Consequently, the taxonomy of the family *Chlamydiaceae* was recently revisited. The genus *Chlamydia* includes currently 11 recognized species, namely *C. abortus* (sheep, goats, cattle), *C. caviae* (guinea-pigs), *C. felis* (cats), *C. muridarum* (mouse, hamster), *C. psittaci* (birds and others), *C. pecorum* (sheep, cattle), *C. pneumonia* (human and others), *C. suis* (swine), *C. trachomatis* (human) and two recently established species isolated from birds, *C. avium* and *C. gallinacea* (75), (Table 24.1).

Table 24.1. *Chlamydiaceae* taxonomy.

Chlamydial taxonomy in the late 1990s			**Chlamydial taxonomy used in the 21st century (Everett et al., 1999) (33)**				**Chlamydial taxonomy used in the 21st century (Stephens et al., 2009) (106)**	
Order	*Chlamydiales*		*Chlamydiales*				*Chlamydiales*	
Family	*Chlamydiaceae*		*Chlamydiaceae*, *Simkaniaceae, Parachlamydiaceae*, *Waddliaceae*				*Chlamydiaceae*, *Simkaniaceae, Parachlamydiaceae, Waddliaceae*	
Genus	*Chlamydia*		*Chlamydia*		*Chlamydophila*		*Chlamydia*	
Species	*C. trachomatis*	Trachoma biovar	*C. trachomatis*	Trachoma biovar			*C. trachomatis*	Trachoma biovar
		LGV biovar		LGV biovar				LGV biovar

		Murine biovar	*C. muridarum*				*C. muridarum*	
		Porcine biovar	***C. suis***				***C. suis***	
	C. pneumoniae	Human biovar			***C. pneumoniae***	TWAR biovar	***C. pneumoniae***	TWAR biovar
		Koala biovar				Koala biovar		Koala biovar
		Equine biovar				Equine biovar		Equine biovar
	C. psittaci	Avian subtype			***C. psittaci***		***C. psittaci***	
		Abortion subtype			***C. abortus***		***C. abortus***	
		Feline subtype			*C. felis*		*C. felis*	
		Guinea-pig subtype			*C. caviae*		*C. caviae*	
	C. pecorum				***C. pecorum***		***C. pecorum***	

Morphology

The 4 morphologically distinct forms of chlamydia are termed elementary body (EB), reticulate body (RB), intermediate body (IB) and the persistent aberrant body. The EB (Figure 24.1) is a small, electron-dense, spherical body, about 0.2–0.3 mm in diameter. The EB is the infectious form of the organism, which attaches to the target epithelial cell and gains entry. The EBs have a highly electron-dense nucleoid located at the periphery of the EB and clearly separated from an electron-dense cytoplasm. Following entry into the host cell, the EB expands in size to form the RB, which is the intracellular, metabolically active form. The RB measures approximately 0.5–2.0 mm in diameter (Figure 24.2). The RB divides by binary fission and thereafter matures into new EBs. During this maturation, morphologically intermediate forms (IB), measuring about 0.3–1.0 mm in diameter, can be observed. The IB has a central electron-dense core with radially arranged individual nucleoid fibers surrounding the core. Cytoplasmic granules are tightly packed at the periphery of the IB and are separated from the core by a translucent zone.

Chlamydiaceae also can engage in a long-term relationship with the host cell, a phenomenon known as persistence, in which no visible growth of the chlamydial organisms can be observed. The normal developmental cycle can be interrupted *in vitro* by a number of conditions and agents, such as antibiotics, nutrient deprivation, or immune factors, interferon-gamma (IFN-γ) in particular. This is generally accompanied by the development of relatively small inclusions, enlarged pleiotrophic RBs, which are named aberrant bodies, and inhomogeneity of the inclusions. Aberrant bodies accumulate chromosomes, but genes for cell division are no longer expressed. Once the stress-

inducing factor is removed, aberrant bodies revert to normal RBs, complete the developmental cycle, and generate infectious EBs

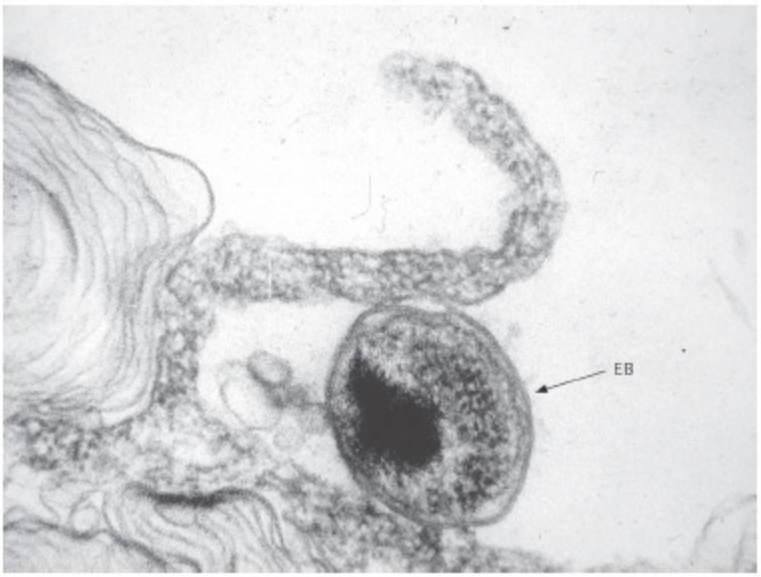

Figure 24.1. Buffalo green monkey (BGM) cell culture, 1 hour after inoculation with the *C. psittaci* Texas Turkey genotype D strain showing an elementary body (EB) attached to the side of a host cell microvillus.

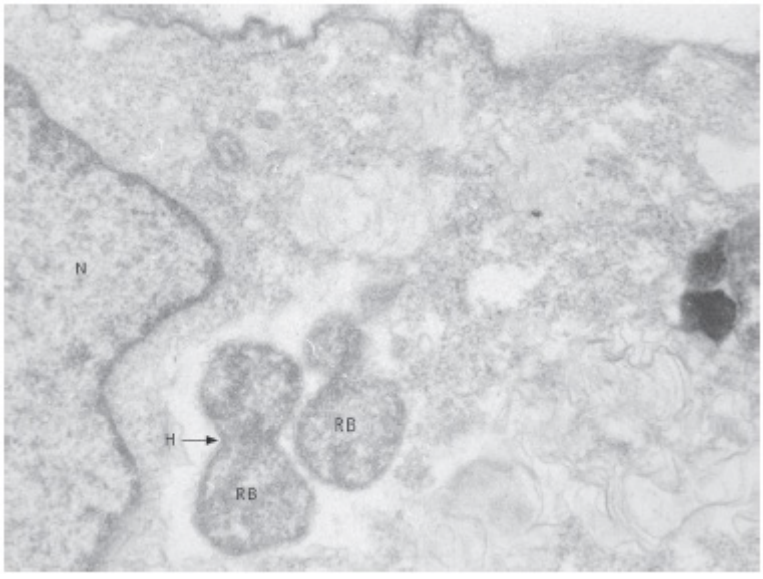

Figure 24.2. Buffalo green monkey (BGM) cell culture, 18 hours after inoculation with a *C. psittaci* genotype B strain (89/1326). Note the vacuole near the nucleus (N) with an early and late stage of division of a reticulate body (R). Note the “hour-glass” profile (arrow H).

Antigenic Structure

The cysteine-rich major outer membrane protein (MOMP) is well studied. It has a molecular weight of 40 kDa and represents approximately 60% of the weight of the outer membrane. The MOMP of *C. psittaci* is an immunodominant protein, and there is considerable evidence that antibodies to surface-accessible epitopes of MOMP have a protective role in immunity to chlamydial infection (23). The outer membrane protein A (*ompA*) gene (formerly referred to as *omp1* gene) encodes the MOMP. The *ompA* gene contains 5 conserved- and four variable-sequence regions, VS1–VS4, which encode for the variable-protein domains VDI–VDIV. VDI, VDII, and VDIV especially protrude from the *C. psittaci* membrane. Epitope mapping has shown the presence of genus- and species-specific antigenic determinants within the conserved regions. However, species-specific antigenic determinants also have been found in the most conserved parts of VDIV. Serovar-specific antigenic determinants are located within VDI and VDII. Monoclonal antibodies to the highly immunoaccessible serovar-specific epitopes on the MOMP can passively neutralize chlamydial pathogenicity and infectivity. Monoclonal antibodies to genus-, species-, or serovar-specific epitopes on the MOMP are excellent tools for specific chlamydial diagnosis.

A chlamydial cysteine-rich heat shock protein 60 (hsp60 or GroEL) has been described that is cross-reactive with other Gram-negative bacteria such as *Escherichia coli*, *Neisseria gonorrhoeae*, and *Coxiella burnetii* (134). Thus, the presence of cross-reactive epitopes on the chlamydial outer membrane should be kept in mind when choosing or interpreting a specific diagnostic test.

Other chlamydia proteins under study are OmcA (EnvA or Omp3), OmcB (EnvB or Omp2), Hc1, RpoB', RpoB, PorB (OmpB), Omp85, elongation factor Tu (EF-Tu/TufA), DnaK (hsp70), OprB, heat shock proteins (10, 44, 58, 59, 61, 86, 93) and especially the recently discovered polymorphic outer membrane proteins (Pmps).

Whole-genome sequencing has revealed the polymorphic membrane protein (Pmp) gene family. This is the largest protein family of *Chlamydia* species and it is a unique feature of the genus (40, 98, 103). Grouping of those proteins in one family is based on the conserved motifs FxxN and GGA (with I, L or V in the 4^{th} position). The Pmps have been identified as autotransporter (type V secretion system) proteins, based on their cleavable N-terminal signal sequence (type II secretion) for translocation across the inner membrane, a central passenger domain which is responsible for the protein's function and a C-terminal transporter domain that forms a β-barrel and with a phenylalanine at the end, which is suggestive for outer membrane localization, for translocation across the outer membrane (reviewed in 120). The Pmp may be involved in antigenic variation and contribute to immune evasion in the infected host. Recently, Van Lent et al., (104), studied the expression all 17 *C. psittaci pmp* coding sequences of the Cal10 strain during both normal and persistent culture conditions. They also used immunofluorescence staining and immuno-electron microscopy. PmpA and PmpH emerged as important players in *C. psittaci* pathogenesis by virtue of their unique expression properties, both at the transcript and protein level.

The chlamydial lipopolysaccharide (LPS) also is an essential constituent of the outer membrane and, like the MOMP, represents one of the major surface-exposed antigens of chlamydiae in both the EB and the RB. It has a molecular weight of 10 kDa and is chemically and serologically related to the LPS of Gram-negative *Enterobacteriaceae*. In

fact, the chlamydial LPS contains several antigenic determinants cross-reacting with the LPS of enterobacterial Re mutants of *Salmonella* species and *Acinetobacter calcoaceticus* (11, 62). However, the chlamydial LPS contains in its saccharide moiety a trisaccharide of 3-deoxy-D-manno-2-octulosonic acid (Kdo) of the sequence αKdo(2→8)-αKdo-(2→4)-αKdo. This antigenic epitope is shared only by all members of the genus *Chlamydia* and, thus, represents a *Chlamydiaceae*-specific antigen useful for specific diagnosis (12).

Strain Classification

Antigenicity

All *Chlamydiaceae* are recognized by monoclonal antibodies (MAbs) that detect the LPS αKdo(2→8)-αKdo-(2→4)-αKdo. *Chlamydia* species have a common antigenic epitope in variable segment 4 of the MOMP: NPTI, TLNPTI, LNPTIA, or LNPTI. *C. psittaci* strains are recognized by serovar-specific monoclonal antibodies. The 8 known *C. psittaci* serovars (A–F, M56, and WC) can be distinguished by use of a panel of serovar-specific monoclonal antibodies in a micro-immunofluorescence test (4, 117). However, serotyping is currently seldom performed because the serovar-specific MAbs are not provided by a commercial supplier and because serotyping appears less discriminatory compared to the newly developed molecular characterization methods (28).

Genetic, Molecular

Chlamydia species can be distinguished by analysis of: (1) full-length 16S and 23S rDNAs, (2) the 16S-23S intergenic spacer (*rrn* spacer), (3) signature sequences in the 16S and 23S ribosomal genes (25, 26), (4) the *Chlamydiaceae* RNase P RNA gene (*rnpB*) encoding a ribonucleoprotein complex that removes 5' leader sequences from tRNA precursors during tRNA biosynthesis (38), or (5) the outer membrane protein A (*ompA*) gene encoding the chlamydia major outer membrane protein (MOMP). Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), nested (multiplex) PCR, TaqMan-based real-time PCR, micro arrays, PCR followed by high resolution melt (HRM) curve analysis of the amplified 16S rRNA gene, as well as gene sequencing has been described for identifying *Chlamydia* species in livestock (26, 29, 57, 73, 78, 79, 80, 109).

OmpA genotyping by real-time PCR using genotype-specific probes is very often used. It allows the detection of an additional variant described as the avian *C. psittaci* genotype E/B (28). A few years ago, a genotyping micro array was introduced, allowing the identification of all currently known avian and mammalian *C. psittaci* genotypes (98). Genotyping is very convenient because it is a rapid, powerful technique that can be used directly on clinical samples in any veterinary clinical laboratory.

Some avian *ompA* genotypes appear to occur more often in a specific order of birds. Genotype A, for instance, is endemic among psittacine birds (*Psittacidae*) but it also has been found in turkeys, ducks, pigeons, and *Passeriformes*. Genotype B is endemic in pigeons (*Columbiformes*) but also can infect chickens, turkeys, ducks, *Psittacidae*, and *Passeriformes* (4). Waterfowl (*Anseriformes*), such as ducks and geese, most frequently seem to be infected with genotype C. Genotype C also has been detected in chickens, ducks and pigeons (22, 42, 136). Genotype D strains are most often associated with

turkeys, but they can also infect pigeons. More recently, genotype D has been detected in chickens (21). Genotype E, also known as Cal-10, MP, or MN, was first isolated during an outbreak of pneumonia in humans during the early 1930s. Later on, genotype E isolates were obtained from a variety of bird species including turkeys, pigeons, ducks, ostriches, and rheas. Genotype F is represented by the psittacine isolates VS225, Prk Daruma, 84/2334 (110), and 10433-MA, but has also been isolated on a Belgian turkey farm (108). Genotype E/B is often found in ducks, but it has also been detected in parrots (35), turkeys (100), and pigeons (30). The mammalian M56 and WC genotypes were isolated during an outbreak in muskrats and hares and during an outbreak of enteritis in cattle, respectively. Subgroups for three of the more heterogeneous genotypes have been introduced, i.e. A-VS1, A-6BC, A-8455, EB-E30, EB-859, EB-KKCP, D-NJ1, D-9N, and provisional genotypes to cover the strains that were previously non-typable have been suggested (77). All genotypes should be considered to be readily transmissible to humans.

Pannekoek et al. (66) used multi-locus sequence typing (MLST) for studying the population structure of *C. psittaci* and *C. abortus* because Van Loock et al. (110) showed that *ompA* sequencing and even sequencing of the *rrn* spacer (25, 26) cannot always distinguish *C. psittaci* from *C. abortus*. The obtained MLST scheme was based on the partial sequences of 7 housekeeping genes, *enoA*, *fumC*, *gatA*, *gidA*, *hemN*, *hflX* and *oppA*, representative for the whole genome sequence. MLST of *C. psittaci* strains resulted in 11 unique sequence types (STs). MLST was extremely useful for distinguishing the phylogenetic highly related species *C. psittaci* and *C. abortus*. Interestingly, according to MLST, the *ompA* genotype F strain 84/2334 appears to be a *C. abortus* strain instead of a *C. psittaci* strain (66).

According to Wang et al. (125), high-resolution genotyping within one chlamydia species can be achieved by multi-locus variable-number tandem-repeat (VNTR) analysis (MLVA) in combination with *ompA* sequencing (MLVA-*ompA*). This method is highly accurate for distinguishing closely related strains within one *Chlamydia* species. VNTR analysis was used for exploring the diversity of *C. psittaci*. For *C. psittaci*, 20 selected genetic loci were initially tested on 9 avian reference strains including representatives of all major *ompA* genotypes (A to F and E/B). Thereafter, 8 loci were retained for a more complete study performed on more than 150 *C. psittaci* isolates from different bird species and geographical origins. The MLVA system provides an additional level of discrimination within the *C. psittaci* species, with 20 distinct patterns identified to date (50). MLVA could provide the high resolution needed for local epidemiology and accurate contact tracing in cases in which zoonosis is contracted from poultry. However, current MLVA (and MLST) methods are still easier to perform on culturable samples or clinical samples of *C. psittaci*- infected poultry with a high bacterial DNA load.

Conserved synteny, i.e. sequence and gene order conservation, in a genome of reduced size is recognized as a hallmark of the genus *Chlamydia* (31). Comparative genomics of *C. psittaci* has already revealed a number of characteristic features (15, 71, 105, 128, 136).

Pathogenicity

Chlamydia psittaci strains isolated from birds fall into two general categories: (1) Highly virulent strains that cause acute epidemics in which 5%–30% of affected birds die and (2)

less virulent strains that cause slowly progressive epidemics. Strains of both high and low virulence appear to have equal ability to spread rapidly through a flock, as evidenced by serologic test results. Highly virulent strains are isolated often from turkeys, ducks, pigeons, and more recently also chickens. They also appear in clinically normal wild birds.

Highly virulent strains have been characterized in European turkeys (118), ducks (48, 96) and more recently also in chickens (49,132). So far, genotypes B, C, F and E/B have been found in chickens (22, 27, 136, 137).

Highly virulent strains cause rapidly fatal disease in natural and experimental hosts with lesions characterized by extensive vascular congestion and inflammation of vital organs. Highly virulent strains have a broad spectrum of pathogenicity for laboratory animals and can cause serious human infections (some fatal) in poultry handlers and laboratory research workers. Strains of low virulence cause slowly progressive epidemics with a mortality rate of less than 5% when uncomplicated by secondary bacterial or parasitic infection. Strains of this category are often isolated from pigeons and are also found in ducks, turkeys, chickens, sparrows, and other wild birds. The turkey isolates from outbreaks with low mortality have been of genotype B or E. Birds infected with these strains usually do not develop the severe vascular damage typical in birds infected with the virulent strains, nor do they have the severe clinical signs (94).

Virulence Factors

The initial event in the infectious process begins with attachment of *C. psittaci* EBs to microvilli at the apical surface of a susceptible columnar epithelial cell (39) (Figure 24.1). The EB travels down the microvillus and locates in indentions of the eukaryotic plasma membrane, some of which resemble coated pits. The bases of micropilli represent areas of active transport of extracellular materials into the cells and, therefore, might assist rapid and efficient entry of EBs. After 1–3 hours, the EBs are internalized in invaginations of the plasma membrane. Uptake of *C. psittaci* is an endocytic mechanism, involving microfilament-dependent and/or independent processes. The *C. psittaci* containing endocytic vesicles or vacuoles escape interaction with lysosomes and proceed in about 8–12 hours to the nuclear area, where EBs are converted to RBs. Conversion to RBs primarily involves reduction of disulfide bond cross-linking among the outer membrane proteins altering the EB cell wall. Synthesis of DNA, RNA, and proteins is initiated, permitting growth and binary fission of the RBs. Binary fission is characterized by the appearance of typical “hourglass” profiles inside the vacuole (Figure 24.2). The enlarging vacuole also is termed an “inclusion.”

C. psittaci microorganisms do not always remain within the inclusion throughout their intracellular development. In some cases, and in apparent correlation with high virulence of strains, the inclusion membrane seems to degrade during the active multiplication, liberating the bacteria into the cytoplasm of the host cell (111). About 30 hours after internalization of the EB, the first RBs are reorganized into newly formed EBs. At about 48–50 hours, the developing chlamydial inclusion may contain anywhere from 100–500 progeny, depending on the characteristics of the *C. psittaci* strain (Figure 24.3). With most *C. psittaci* strains, the host cell has undergone severe degenerative changes, and microorganisms are released by lysis (Figure 24.4). Exocytosis of the inclusion, followed by a “healing” or closing of the open-cavern structures where the inclusion had existed,

has been reported (111). Persistent infections may occur with non-replicating RBs, the so called aberrant bodies, remaining inside the host cell cytoplasm.

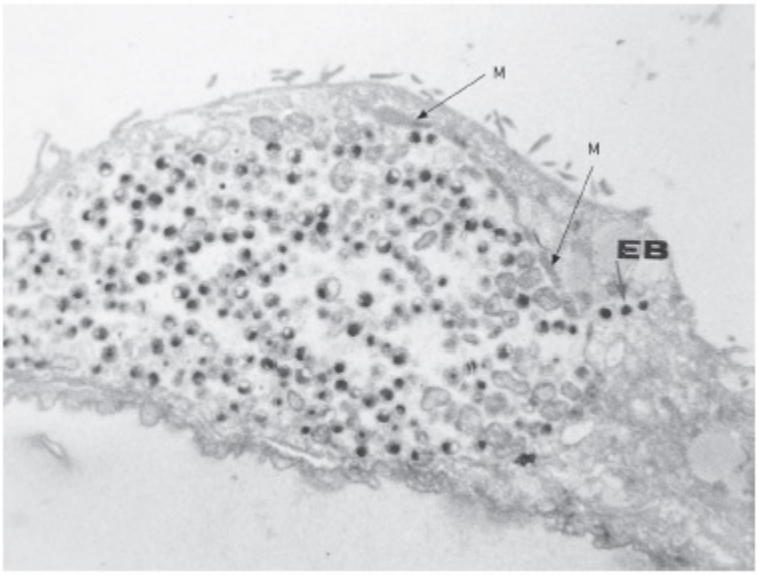

Figure 24.3. BGM cell culture, 52 hours after inoculation with a *C. psittaci* genotype D strain (92/1293), isolated from diseased turkeys. Note the large inclusion and the elementary bodies (EB) apparently "escaping" from the inclusion. Also notice the mitochondria (M) lining the inclusion.

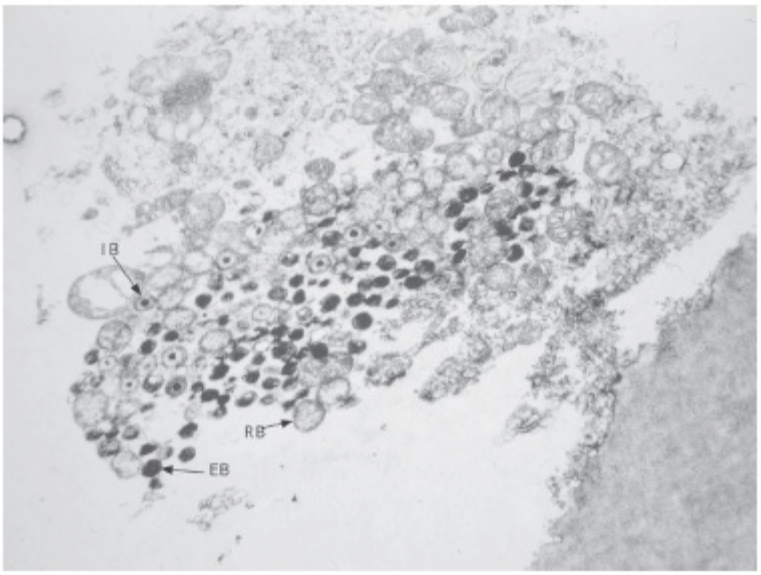

Figure 24.4. BGM microcarrier culture, 50 hours post inoculation with the *C. psittaci* Texas Turkey genotype D strain showing lysis of an infected BGM cell. The cell is sloughing off the microcarrier (MC). Note the presence of elementary bodies (E), reticulate bodies (R), and intermediate bodies (I).

Long, unique, rosette-like structures and projections have been observed on the surface of both EBs and RBs of *C. psittaci* strain Mn (56). Shortly after the first description of a type III secretion system (T3SS) in *C. caviae* (GPIC strain) (41), Bavoil and Hsia (5) speculated that Matsumoto's projections are in fact functional T3SSs,

injecting chlamydial virulence proteins into the host cell cytoplasm. *Chlamydia psittaci* strains also contain a type three secretion system (T3SS) (8). Beeckman et al., (8) identified a T3SS in a virulent *C. psittaci* genotype D strain isolated from turkeys and studied its possible role in virulence.

Outer virulence factors under study are the polymorphic membrane proteins (Pmps) (93). The Pmps belong to the family of autotransporter proteins (type V secretion system). Most autotransporter proteins contribute to the virulence of many Gram-negative pathogens. Specific roles in pathogenesis have been described, including adhesion, host and tissue tropism and antigenic variation (immune evasion) (6, 58, 92, 126). Additional potential functions of the Pmp proteins still need to be examined.

Pathobiology and Epidemiology

Incidence and Distribution

Avian chlamydiosis occurs worldwide, with the incidence and distribution varying greatly with the species of bird and the genotype of the chlamydial organism. Antibiotics have been used extensively to control the spread of the disease in birds and to reduce the risk to humans.

The disease pattern in turkeys has changed. Historically, most outbreaks were explosive and occurred in free-ranging birds. These severe respiratory disease outbreaks were attributed to genotype D. Chlamydia was thought to have been introduced from the outside. Today, genotype D and sometimes genotype A are found in confinement turkeys when death losses are high; genotype B is less virulent (4, 74). Introduction through vertical transmission (55, 127) or through infected hatchlings (20) may occur. Studies on turkeys in Belgium and France showed that strains (genotypes A, B, D, E, E/B, and F) of high and low virulence are widely distributed in commercial turkeys and may be endemic. Chlamydia is part of the turkey respiratory disease complex (108). Infection of turkey broilers with *C. psittaci* also seems to predispose animals to more severe clinical outcomes of a simultaneous or subsequent infection with *Escherichia coli*, the avian metapneumovirus and/or *Ornithobacterium rhinotracheale* (106, 107, 108).

Over the last decade, *Chlamydia psittaci* infections in ducks seem to be reported more often in China and Europe (14, 16, 32, 52, 97, 131, 132) than in the US. In Europe, the number of outbreaks in ducks seems to be increasing as well as the number of zoonotic case reports linked to handling ducks (42, 48, 123). This could be due to reduced antibiotic use in poultry or to the occurrence of more virulent *C. psittaci* strains. European isolates have been characterized as genotype C and genotype E/B (28, 42, 48). In China, genotypes A and C have been found in ducks (83, 131).

C. psittaci outbreaks on chicken farms and zoonotic transmissions linked to contact with *C. psittaci*-infected chickens have also appeared to occur more frequently over the last decade (17, 20, 21, 27, 33, 46, 49, 73, 131, 132). In one study by Verminnen at al (122) the author investigated the occurrence of *C. psittaci* by performing a retrospective study of 300 serum samples collected in 2005 from 10 randomly selected chicken breeder, broiler, and layer farms in Belgium. Sera were examined using a recombinant MOMP-based enzyme-linked immunosorbent assay (ELISA). Seropostive results were obtained from 98%, 95%, and 95% of layers, broilers, and breeders, respectively (21),

and seropositive birds were found on all farms. Highly virulent genotype A and D strains, as well as genotypes B, C, F and E/B have been found in chickens in Belgium, China, France, and Germany (27, 46, 49, 132, 135, 137).

In 2008, 3 cases of atypical pneumonia in individuals working at a French slaughterhouse processing guinea fowl, ducks, and especially chickens prompted an epidemiologic survey of the 10 farms that had supplied the birds. Using a *Chlamydiaceae*-specific real-time PCR assay, chlamydial agents were detected in 14 of the 25 investigated flocks. In one duck flock studied, 20% of the tested animals were positive. Additionally, 12 of 18 (67%) and 1 of 6 (17%) of the chicken and guinea fowl flocks examined were PCR positive, respectively. Positivity for the chicken flocks ranged from 10%-100%. For the positive guinea fowl flock, 12.5% of the tested animals were positive. Rather unexpectedly, *Chlamydia psittaci* was identified only in the positive duck flock, whereas ArrayTube DNA microarray testing indicated the presence of a new chlamydia agent in of all the other French poultry flocks that were examined. Further studies on the agent found in chickens revealed the presence of a new member of the family *Chlamydiaceae* namely *C. gallinacea* sp. nov. (49, 75).

Data on *C. psittaci* infections in meat-type pigeons are primarily published in China. An indirect haemagglutination assay (detects *Chlamydiaceae*) was used to examine the seroprevalence of *C. psittaci* infections in Guangdong. Seroprevalence was 17% (34/200) in meat-type pigeons obtained from seven commercial flocks (53). Occupationally contracted psittacosis was reported after contact with meat-type pigeons in Beijing. Employees of pigeon farms in Beijing were diagnosed as psittacosis by positive CFT and recovered after treatment with erythromycin (131).

Natural and Experimental Hosts

Chlamydiae or chlamydial antibodies have been found in at least 465 bird species (43). Common reservoirs of chlamydiae include wild and feral birds such as sea gulls, ducks, Canada geese, herons, egrets, pigeons, blackbirds, grackles, house sparrows, and killdeer, all of which freely intermingle with domestic birds (51). Highly virulent strains of *C. psittaci* can be carried by and excreted in large numbers without any apparent effect on these hosts.

Experimental hosts of avian chlamydiae can include virtually any species of bird. Mammalian laboratory hosts used for avian chlamydiae are principally mice and occasionally guinea pigs. Mice and guinea pigs are the natural hosts for *C. muridarum* and *C. caviae*, respectively. Investigators using these animals should determine the chlamydial status of the breeding stock. Rabbits are refractory to clinical disease caused by avian chlamydiae, but they may be used to produce polyclonal antibodies.

Younger domestic birds generally are more susceptible than older birds to infection, clinical disease, and mortality. However, maternal antibodies might protect against respiratory disease outbreaks on the farm. Infection in old turkeys, such as spent breeder hens, can go unnoticed unless birds are subjected to stressful conditions such as shipment to market on crowded trucks. Turkey toms may have a higher mortality rate than turkey hens.

Transmission, Carriers, and Vectors

Transmission of *C. psittaci* primarily occurs from one infected bird to another susceptible bird in close proximity. The agent is excreted in feces and nasal discharges. Fecal shedding occurs intermittently and can be activated through stress caused by nutritional deficiencies, prolonged transport, overcrowding, chilling, breeding, egg laying, treatment, or handling. Bacterial excretion periods during natural infection can vary depending on virulence of the strain, infection dose, and host immune status. However, shedding may occur for several months. Transmission of chlamydiae occurs primarily through inhalation of contaminated material and, sometimes, ingestion.

Large numbers of *C. psittaci* cells can be found in respiratory tract exudate and fecal material of infected birds. The importance of the respiratory exudate in transmission has become more apparent. In turkeys, the lateral nasal glands become infected early and remain infected for more than 60 days. Choanal/oropharyngeal swabs are more consistent for isolation of the agent than fecal swabs, especially during early stages of infection. Direct aerosol transmission through aerosolization of respiratory exudate must be considered as the primary method of transmission

Avian species, including domestic poultry sharing aquatic or moist soil habitats with wild infected aquatic birds, may become infected via contaminated water. Granivorous birds, like pigeons, doves, pheasants, and house sparrows, may become infected by dust inhalation in barnyards and grain storage sites contaminated by feces. The consumption of infected carcasses may transmit *C. psittaci* to host species that are predators or scavengers of other birds.

Transmission of *C. psittaci* in the nest is possible. In many species, such as *Columbiformes*, cormorants, egrets, and herons, transmission from parent to young may occur through feeding by regurgitation, while contamination of the nesting site with infective exudates or feces may be important in other species, such as snow geese, gulls, and shorebirds. Furthermore, *C. psittaci* can be transmitted from bird to bird by bloodsucking ectoparasites such as lice, mites, and flies or, less commonly, through bites or wounds. Transmission of *C. psittaci* by arthropod vectors may be facilitated in the nest. Mites from turkey nests can contain chlamydiae (23) and during an epidemic in turkeys in South Carolina, simulid flies were suspected as a possible method of transfer (64). Transmission through insects is indeed not unlikely as a recent study by Pilloux et al., (68), demonstrated a high prevalence and variety of *Chlamydiales* DNA within *Ixodes ricinus* ticks.

Vertical transmission has been demonstrated in turkeys, chickens, ducks, parakeets, seagulls, and snow geese, although the frequency appears to be fairly low (55, 127). However, it could serve as a route to introduce chlamydiae into a poultry flock. Recently, experimental evidence was presented for the transmission of *C. psittaci* in poultry by eggshell penetration (1).

Chlamydia psittaci can be introduced into poultry through the wild bird population. Contaminated feed, barn bedding or equipment also can be a source of infection, and feed should therefore be protected from wild birds. Careful cleaning of equipment being used in several barns during one and the same production round is extremely important because *C. psittaci* can survive in feces and bedding for up to 30 days (34).

Incubation Period, Clinical Signs, Morbidity and Mortality, Pathology, and Pathogenesis

Turkeys

Vanrompay et al. (113) used immunodetection to study the pathogenesis of *C. psittaci* genotype A, B, and D strains in specific pathogen free turkeys. Use of immunodetection allowed precise determination of tissue and cell tropism. In this study, turkeys were aerosol infected, because it represents the natural route of infection (65). From this study, the following pathogenic sequence of events can be deduced for all three genotypes investigated. In turkeys infected by aerosol, the primary site of replication is the upper respiratory tract, where epithelial cells become infected. Subsequently, epithelial cells of the lower respiratory tract and macrophages throughout the respiratory tract become infected. Then, intense replication occurs in the respiratory tract. At the same time, chlamydiae can be demonstrated in plasma and monocytes, indicating septicemia, and chlamydiae appear in epithelial cells and macrophages of various tissues throughout the body.

In turkeys, an experimental infection with a genotype B strain induced much milder clinical signs and lesions than infection with genotype A or D strains (115). The genotype B strain had a longer incubation period, took longer to reach maximum titers in the tissues, and had shorter periods during which the organism was found in the tissues.

The incubation period of chlamydiosis in naturally infected birds varies, depending upon the number of chlamydiae inhaled, the virulence or pathogenicity of the infecting strain for that host species, and host immunogenetics. Experimentally, definitive disease signs in young turkeys receiving a virulent strain may be evident in 5–10 days. In birds naturally exposed to smaller doses or in older birds, the period may be longer. Strains of lower virulence, which cause less severe signs, may have longer incubation periods. Clinical signs may not be noticeable until 2–8 weeks after exposure.

Signs of chlamydiosis in turkeys infected with virulent strains are cachexia, anorexia, elevated body temperature, conjunctivitis, and respiratory distress. Diseased birds excrete yellow-green, gelatinous droppings. Egg production of severely affected hens declines rapidly to 10%–20% and may temporarily cease or remain at a very low rate until complete recovery. Disease signs in a flock infected with strains of low virulence are usually anorexia and loose, green droppings in some birds, with less effect on egg production.

At the peak of disease outbreak in a flock infected with a virulent strain, 50%-80% of the birds will show clinical signs, whereas morbidity from less virulent strains is only 5%-20%. Mortality caused by virulent chlamydia ranges from 10%-30% and is only 1%-4% with less virulent strains.

The less virulent strains cause gross lesions, which are similar to those caused by virulent strains, only less severe and extensive. In overwhelming infections with virulent strains, lungs show diffuse congestion, and the pleural cavity may contain fibrinous exudate. In fatal cases, a dark transudate may fill the thoracic cavity. The pericardial membrane is thickened, congested, and coated with fibrinous exudate. The heart may be enlarged, and its surface may be covered with thick fibrin plaques or encrusted with yellowish, flaky exudate (Figure 24.5). The liver is enlarged and discolored and may be coated with thick fibrin. Air sacs are thickened and heavily coated with fibrinous exudate (Figure 24.6). The spleen is enlarged, dark, and soft and may be covered with gray-white spots representing areas of focal cellular proliferation. The peritoneal serosa and mesentery show vascular congestion and may be coated with foamy, white fibrinous

exudate. All of these exudates contain large numbers of mononuclear cells in which numerous microcolonies of chlamydial RBs may be seen. Fibrinous exudates, found on all organs and tissues of the thoracic and peritoneal cavities, reflect vascular damage as well as increasing inflammatory response caused by the continued multiplication of the organisms. In birds that survive infection with a strain of low virulence, the lungs may not be seriously affected. However, multiplication of organisms on the epicardium may result in the formation of 1 or more fibrin plaques.

Vanrompay et al. (112) examined histopathologic changes in 4 groups of 20 specific pathogen free (SPF) turkeys kept in isolation units and inoculated by the natural route of infection (aerosol). Turkeys were experimentally infected with strain 84/55 (*C. psittaci* genotype A), isolated from a parakeet, strain 92.1293 from a turkey (*C. psittaci* genotype D), the Texas Turkey strain (*C. psittaci* genotype D), or strain 89/1326 (*C. psittaci* genotype B) from a pigeon. All 4 strains proved to be pathogenic for SPF turkeys. Turkeys showed conjunctivitis, sinusitis, rhinitis, keratitis, pericarditis (Figure 24.5), pneumonia, airsacculitis (Figure 24.6), hepatosplenomegaly, enteritis, congestion of the kidneys, and congestion of the ovaries or testes. There were epithelial erosions and fibrin deposit in the conjunctivae (Figure 24.7), corneal ulceration, bronchopneumonia (Figure 24.8), fibrinous necrotizing airsacculitis (Figure 24.9), fibrinous pericarditis, interstitial nephritis, peritonitis, and catarrhal enteritis. The type and distribution of the lesions was similar for genotypes A and D. However, the lesions produced after genotype A infection appeared more severe. For genotype B, in comparison with both other genotypes, no lesions were observed in the small intestine, pancreas, ovary, and testis.

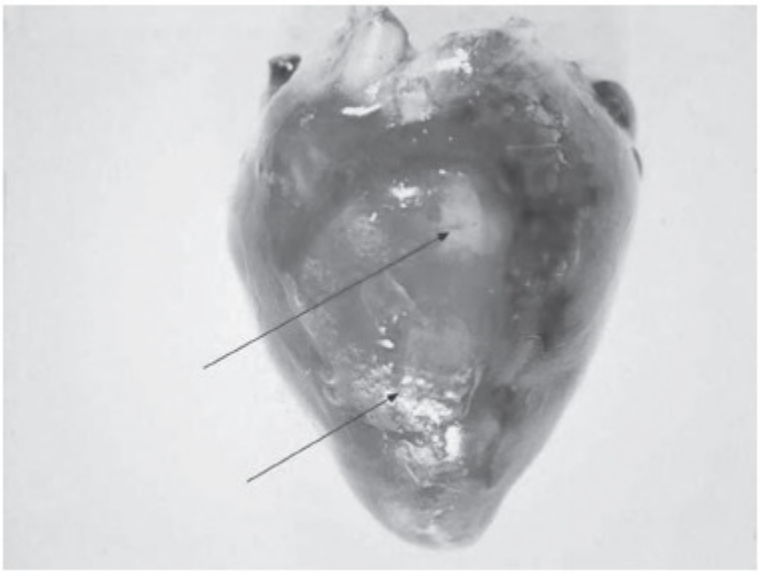

Figure 24.5. Turkey experimentally infected (aerosol) with a *C. psittaci* genotype A strain (84/55) isolated from the lungs of a budgerigar. Note the presence of serous fluid together with fibrin in the pericardial sac.

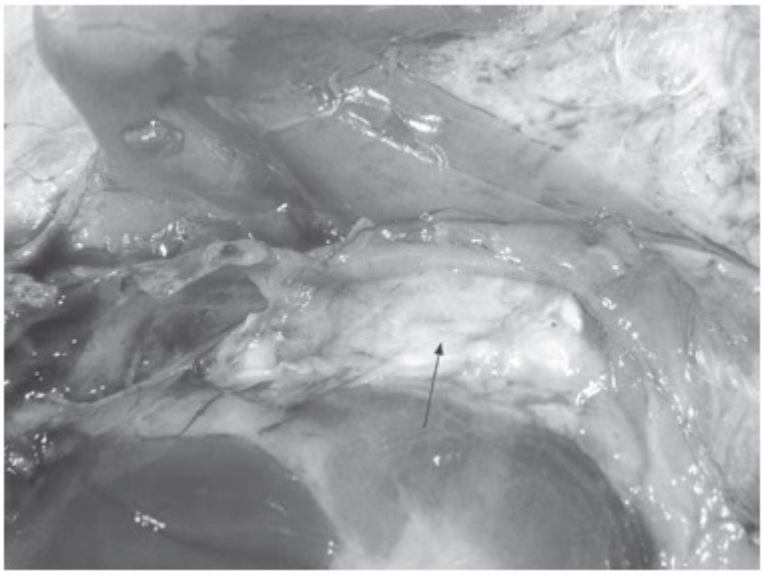

Figure 24.6. Turkey experimentally infected (aerosol) with a *C. psittaci* genotype A strain (84/55). Note the thickened abdominal airsac totally covered with fibrin cloths (arrow).

Figure 24.7. Hematoxylin and eosin staining of experimentally infected turkeys. Conjunctiva with infiltration of lymphocytes and heterophils together with epithelial vacuolization and hyperplasia. ×172.

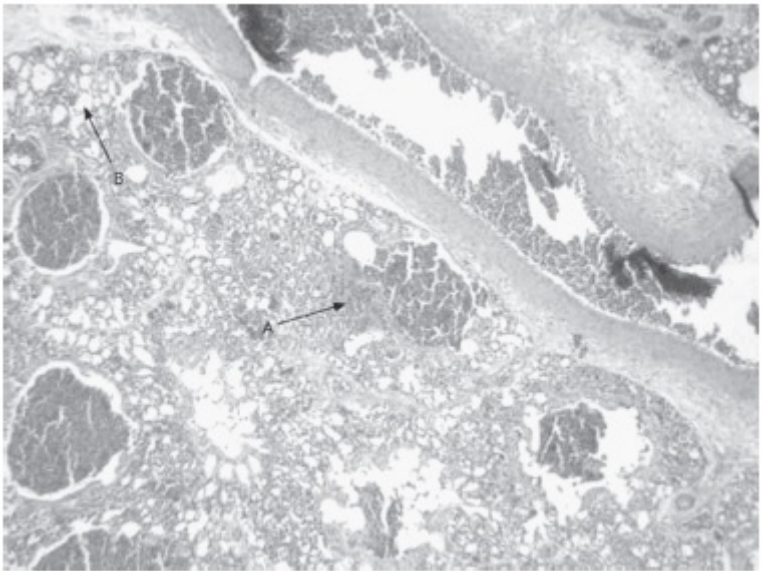

Figure 24.8. Hematoxylin and eosin staining of experimentally infected turkeys. Congested lung with infiltration of lymphocytes (arrow A) and dilated bronchi and parabronchi (arrow B). ×69.

Controlled dual infections in SPF turkeys demonstrated the pathogenic interplay between *C. psittaci*, avian metapneumovirus, and *E. coli*. *Escherichia coli* is a predisposing factor for the outcome of a *C. psittaci* infection. It can increase the severity of a *C. psittaci* infection and can reactivate a latent *C. psittaci* infection (106). An aMPV infection during the acute phase of a *C. psittaci* infection aggravates the severity of clinical signs, macroscopic lesions, pharyngeal aMPV excretion, and histological tracheal lesions. However, no clear interaction was established after an MPV infection in latently *C. psittaci*-infected turkeys (107).

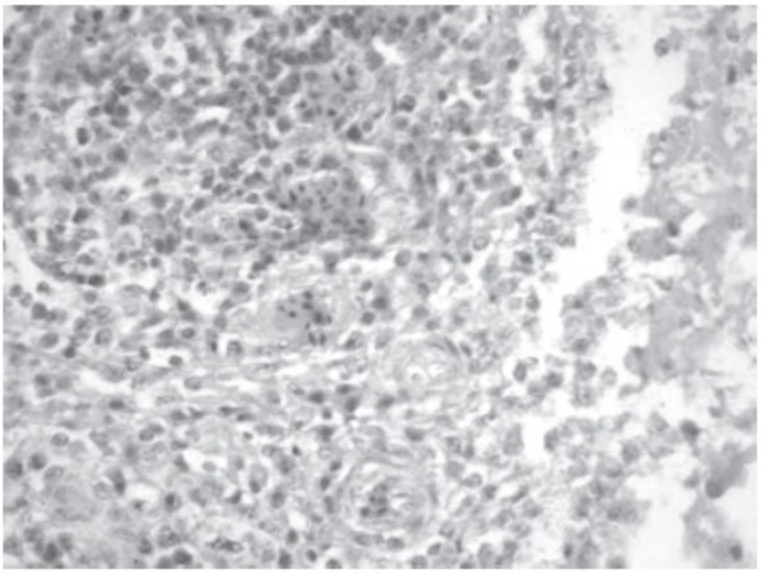

Figure 24.9. Hematoxylin and eosin staining of experimentally infected turkeys. Fibrinous necrotizing airsacculitis. ×172.

Chickens

Epidemiologic evidence formerly indicated that chickens are relatively resistant to disease caused by *C. psittaci*. Acute infection progressing to disease and mortality only occurred in young birds, and the incidence of actual epidemics was very low. Most natural infections in chickens were believed to be inapparent and transient. However, *C. psittaci* strains isolated from turkeys caused similar pathology and mortality in chickens as in turkeys (88, 90).

Recently, highly virulent *C. psittaci* strains have been isolated from the lungs of diseased chickens raised in Belgium, France, Germany, and China (27, 131, 132, 137). The strains obtained from Belgian and French farms belonged to genotypes D and B and could successfully be used to reproduce the disease in experimentally infected SPF chickens (132). Chickens showed conjunctivitis, rhinitis, pneumonia, fibrinous airsacculitis, fibrinous pericarditis, and hepatosplenomegaly. Histopathological lesions (133) and mortality was observed.

Ducks and geese

Chlamydiosis in domestic ducks is important both economically and as a public health hazard. Over the last decade, outbreaks are primarily reported in China and Europe (14, 32, 33, 48, 49, 52, 131). Chlamydiosis in ducks is usually a severe, debilitating, often fatal disease in which young ducks develop trembling, imbalanced gait, and cachexia. They become anorexic with green, watery intestinal contents and develop a serous to purulent discharge from the eyes and nostrils causing the feathers on the head to become encrusted with exudate. As the disease progresses, the ducks become emaciated and die in convulsions. Morbidity ranges from 10%–80%, and mortality varies from 0–30% depending on age and the presence of concurrent infections. Recently, severe outbreaks associated with human disease were reported in France (48, 49).

Incidental to studies of chlamydiosis in ducks, several investigators have observed *C. psittaci* antibodies or the disease in geese and have isolated *C. psittaci* from diseased tissues (2). Clinical disease and necropsy findings were similar to those in ducks.

Pigeons

Signs of uncomplicated chlamydiosis in meat pigeons are variable, but those that develop acute disease are anorexic, unthrifty, and diarrhetic (2, 137). Some develop conjunctivitis, swollen eyelids, and rhinitis. Respiratory difficulty is accompanied by rattling sounds. As disease progresses, birds become weak and emaciated. Mortality occurs. Recovered birds become asymptomatic carriers. Some birds progress through an infection showing no signs or, at the most, transient diarrhea before becoming carriers. Salmonellosis or trichomoniasis exacerbates the illness in chlamydia-infected carrier birds, inducing signs and lesions of acute disease. Gross lesions of uncomplicated chlamydiosis in pigeons are fibrinous exudates on thickened air sacs, the peritoneal serosa, and occasionally the epicardium. The liver is usually swollen, soft, and discolored. The spleen may be enlarged, soft, and dark. Greater than normal amounts of urates are seen in cloacal contents if catarrhal enteritis occurs. In less severe infections, only the liver or air sacs are involved. Some heavily infected shedders have no lesions.

Pheasants, Quail, Guinea Fowl, Peacocks and Partridges

Chlamydiosis has been reported in farm-raised pheasants, quail, peacocks, guinea fowl and partridges from all over the world (24, 42, 131). The clinical signs and lesions are similar to those seen in other birds (91). Morbidity and mortality can be very high, especially in young birds. Only few human infections have been reported during the last decade (42, 49, 130, 131)

Immunity

Natural immunity to chlamydia is generally poor and short-lived. As birds become older, however, they become more resistant to clinical disease, even though infection may occur.

The immune response to chlamydia is a coordinated event in which innate immune cells, B cells and T cells, act in concert and each of these immune effectors have roles in recognizing different stages of the infection. To date, *Chlamydia psittaci* vaccine studies suggest that the ideal, efficacious chlamydia vaccine should induce $CD4^+$ T helper 1 (Th1), and $CD8^+$ cytotoxic T cell responses (70). Humoral immune responses, albeit not considered as crucial, seem to contribute significantly to protection (121).

Less is known about innate immune detection of *C. psittaci*. Beeckman et al. (7) determined the cytokine responses following *C. psittaci* infection of avian monocytes/macrophages. High IL-10 and no TGF-b responses were observed at 4 hours post inoculation. This could induce macrophage deactivation and NF-kB suppression, and thereby could dampen innate immunity and promote *C. psittaci* survival in macrophages.

Diagnosis

The preferred method for the identification of AC is no longer isolation and identification of the organism. Because of the time involved, the need for high-quality samples, the fact that some strains will never grow *in vitro* and the hazard to laboratory personnel (BSL3 laboratory required), nucleic acid amplification tests (NAAT's) are currently recommended for quick, sensitive and specific diagnosis. These include conventional and real-time polymerase chain reaction (PCR), DNA microarray-based detection and DNA sequencing. Culture, cytological staining, immunological staining, immunohistochemistry or antigen enzyme-linked immunosorbent assays (ELISA) can be used if NAAT's are not available.

Specimen Collection and Storage of Samples

The following samples should be preferably collected: pharyngeal/choanal slit swabs in live birds experiencing respiratory disease (3, 112) and/or conjunctival swabs if indicated by the presence of conjunctivitis. Cloacal swabs or fresh feces are less optimal because chlamydial shedding is intermittent. In dead birds, especially lungs and thickened exudate-coated air sacs are most suitable but spleen, liver and free exudates can also be sampled.

Specimens should be collected aseptically if willing to culture chlamydiae as contaminating bacteria can interfere with the isolation of chlamydiae. Proper handling of clinical samples is necessary to prevent loss of infectivity if culture is to be used. If

specimens are used to inoculate cell cultures or embryonated eggs immediately, most diluents will be adequate; however, if the specimen is to be shipped and/or stored before analysis, a special chlamydia transport medium should be used (84,119). Samples in chlamydia transport medium can be stored for 1 or 2 days at 4°C prior to analysis. However, longer preservation needs to be done at -80°C. Chlamydiae in tissue specimens or yolk-sac suspension can be preserved almost indefinitely by storage at -80°C.

Culture

Preparation of Inoculum

Prior to inoculation, samples must be treated properly. The processing of samples is similar for inoculation of cell cultures or embryonating eggs. Penicillin and tetracyclines should be avoided because they inhibit the growth of *C. psittaci*.

A standard procedure is to prepare 20% tissue suspensions in phosphate bufferend saline (PBS). The suspensions are centrifuged (2,790 × g, 4°C) for 10 minutes. The supernatants are collected and 10 µl/ml streptomycin sulfate (streptomycin sulfate 1% w/v) and 20 µl/ml vancomycin (vancomycin 0.5 w/v) are added. If needed, 0.1% amphotericin B can be added. After 1 hour of incubation at room temperature, the suspensions are centrifuged for 30 minutes (2,790 × g, 4°C) and supernatants are immediately used for inoculation or stored at –80°C until use. Swabs in chlamydia transport medium are shaken for 1 hour at 4°C on a rocking platform, centrifuged (2,790 × g, 4°C), and the supernatant immediately used or stored at –80°C until use. Fecal samples, although not preferably used for diagnosis because of the intermittent chlamydia shedding and the risk for false negatives, are processed as follows. A 20% suspension is made in PBS, and the suspension is shaken for 1 hour (4°C) on a rocking platform. Afterward, the suspension is sonicated for 10 minutes using an ultrasonic water bath. The supernatant is collected, and 10 µl/ml streptomycin sulfate (streptomycin sulfate 1% w/v), 20 µl/ml vancomycin (vancomycin 0.5 w/v), and 0.1% amphotericin B are added. The suspension is incubated at room temperature (1 hour) and subsequently centrifuged (2,790 × g, 4°C) for 30 minutes. The supernatant is collected and thereafter ultracentrifuged (45,000 × g, 4°C, 45 minutes). The supernatant is discarded, and the remaining chlamydia pellet is re-suspended in diluent and immediately used for inoculation or stored at –80°C until use.

Cell cultures are the most common and convenient method for the isolation of *C. psittaci*. The most commonly used cell lines are Buffalo green monkey (BGM), Vero, McCoy, HeLa, and L-929, although a number of other cell cultures, such as chicken embryo fibroblasts, can be used. A study showed BGM to be the most sensitive, with Vero and L-929 listed as satisfactory (136). Standard cell culture medium is used, containing 5%–10% fetal calf serum and antibiotics like vancomycin, streptomycin, and amphotericin B because they do not inhibit the growth of *C. psittaci*. Cell culture harvest can be frozen at -80°C in sucrose phosphate glutamate (SPG) buffer (1/1; SPG/culture medium).

The culture equipment must be suitable for: (1) preferably identification by immunofluorescence staining, (2) centrifugation (500-1,500 × g for 60 to 90 minutes) of the inoculum onto the monolayer at 37°C to enhance infectivity (eventually in the

presence of diethylaminoethyl (DEAE), (3) possible blind passages at 3 or 6 days post inoculation to increase sensitivity of isolation, (4) examination of the sample 2–3 times during passage, and (5) protection of humans against possible infection. Small, flat-bottomed vials (1-dram shell vials) or bottles with 12-mm diameter glass coverslips meet these requirements and are often used because the cell culture monolayer can be grown directly on the coverslip. Several vials are inoculated with each sample to permit fixing and staining at various times and to permit passages of negative samples after 6 days of incubation.

Chlamydiae can be isolated from cells that are replicating normally. Most diagnosticians, however, prefer to use nonreplicating host cells for two reasons: (1) to provide increased nutrients for the replication of chlamydiae and (2) because nonreplicating cells can be maintained for longer periods for observation. Host-cell replication is suppressed most commonly using cycloheximide (0.5-2.0 mg/ml). Incubation is usually at 37°C–39°C, depending on the cell culture used. Disruption of the monolayer by freeze-thawing should be avoided because it can destroy *C. psittaci*.

Some laboratories still use chicken embryos for primary isolation of chlamydiae. The standard procedure is to inject up to 0.3 ml of inoculum into the yolk sacs of 6-day-old embryos (136). Replication of chlamydia usually will cause the death of the embryo within 5–12 days after inoculation. If no deaths occur, two additional blind passages are usually made before calling the sample negative. Chlamydia infection typically causes vascular congestion of the yolk-sac membranes, which are harvested and homogenized as a 20% yolk-sac suspension. This suspension can be frozen (-80°C) to preserve the strain or inoculated into other eggs if needed or into cell culture monolayers. *C. psittaci* is usually identified by immunofluorescence staining of yolk-sac impression smears.

Staining Cell Monolayers or Yolk-Sac Impression Smears

The preferred method for fixing of the monolayer is to remove the medium, wash once with PBS, and fix with cold acetone for 10 minutes (-20°C). If the cell culture vessel is plastic, the monolayer can be fixed with a mixture of 50% acetone and 50% methyl alcohol or with 100% methyl alcohol. The preferred method for staining is the fluorescence method. With the fluorescence method, the fluorescein-conjugated anti-chlamydia serum is applied to the glass slide and incubated for minimum of 30 minutes at 37°C. The slides are then washed with PBS and deionized water, air dried, and mounted for use by a fluorescence microscope. Commercially fluorescently labeled monoclonal antibodies are available.

Direct visualization - Cytological staining techniques

Chlamydiae can be detected in smears of cloacal and/or conjunctival swabs and in impression smears of tissues (lung, liver, spleen, kidney and airsacs if enough material is available) by cytological staining such as Giemsa, Giménez, modified Giménez, Ziehl–Neelsen and Macchiavello's stains (13). The modified Giménez technique is most often used (2). However, none of the stains specifically detects chlamydia. They are all less sensitive than antibody-based antigen detection methods or specific NAATs. Therefore, use of a cytological staining is losing popularity.

Antigen detection

Immunological staining techniques

Immunofluorescence staining can be used to detect chlamydiae in smears of cloacal and/or conjunctival swabs, in impression smears of tissues and in frozen tissue sections. Most commercial staining kits use FITC conjugated anti-LPS or anti-MOMP monoclonal antibodies and most of them are originally developed for detection of *C. trachomatis* in human specimens.

Immuno-histochemical staining is used to detect chlamydiae in paraffin sections and to show the association of chlamydial agents and pathological lesions in tissues. Commercial anti-*Chlamydia* antibodies for immunohistochemistry are available and most of them detect the Chlamydial LPS.

Antigen Enzyme-linked immunosorbent assays

The ELISA has been extensively promoted in kit format for use in the diagnosis of human chlamydiosis. These test kits detect the lipopolysaccharide (LPS) antigen (group reactive) and will detect all species of *Chlamydiaceae*. In the past, a number of these kits have been tested for use in detecting chlamydiae in birds (116), but none of the kits has been licensed for detection of *C. psittaci*. One problem with some of these tests is that the chlamydial LPS shares some epitopes with other Gram-negative bacteria, and these epitopes can cross-react, resulting in a high number of false-positive results. This problem has been reduced or eliminated in more recently developed kits by careful selection of the MAbs used. These kits, however, still lack sensitivity because a few hundred organisms are still needed to give a positive reaction.

Detection of nucleic acids

Reagents designed to stabilise the DNA should be considered when a delay in processing the sample is anticipated (18). DNA samples can be prepared using inexpensive reagents or commercially available kits.

In the last decade, real-time PCR has become the preferred method in diagnostic laboratories for its rapidity, high throughput, potential for quantification and ease of standardisation. Several real-time PCR's for the detection of *C. psittaci* have been developed (29, 37, 67). This technology requires a fluorescent-labelled probe and special equipment, which increases costs. Its sensitivity is lower than that of the nested PCR (78, 109) but contamination problems due to post-PCR carry over of DNA of a previous amplification round and labour are reduced as it is based on one reaction in a closed system. *Omp*A-based real-time PCR protocols were developed to differentiate between genotypes of *C. psittaci* (29, 36) and to distinguish *C. psittaci* from *C. abortus* (63). The PCR developed by Heddema et al (36) is also validated on a large number of human psittacosis samples and thus is helpful to trace infection sources of zoonotic transmission. Real-time PCR protocols are available for the specific detection of *C. avium* (139) and *C. gallinacea* (47).

DNA microarray technology has also been used in the diagnosis of chlamydial infections in animals (78). The assay for detection and identification of *Chlamydiaceae* spp. is

based on PCR amplification of the 23S rRNA gene and subsequent identification of *Chlamydia* species occurring in animals, including *C. psittaci*, *C. avium* and *C. gallinacea,* by hybridisation with species-specific probes. An extended version of the *Chlamydiaceae* DNA microarray allows for *omp*A-based genotyping of *C. psittaci* strains (77).

Serology

Serology alone is not particularly useful in diagnosing a current chlamydial infection in birds because of the high prevalence of this infection in birds and the long-term (up to several months) persistence of anti-chlamydial antibodies. In most bird species, there is a high background rate of anti-chlamydial antibodies in birds. Thus, to determine if a single bird is infected, serology should always be used in conjunction with antigen or gene detection, or paired sera should be examined. However, obligatory examination of paired sera removes serology from immediate clinical relevance. A positive test is evidence that the bird was infected by the bacterium but does not necessarily indicate an active infection. False negative results can occur in birds with acute infections that are sampled before seroconversion. Treatment with antibiotics also may delay and/or diminish the antibody response. The main serological method used for detecting chlamydial antibodies is the complement fixation test (CFT). However, the complement fixation test is more often being replaced by highly sensitive and specific ELISA's based on the use of recombinant proteins (122) or peptide antigens (76). ELISA's can detect avian IgM, IgG and IgA as long as the correct isotype-specific conjugate is used.

Differential Diagnosis

The signs of chlamydiosis in birds are non-specific and resemble those observed in many other diseases. Suspected chlamydiosis may have to be differentiated from pasteurellosis, particularly in turkeys, in which some signs and lesions may be similar. Pasteurellosis can be ruled out by appropriate culture procedures. Because of some similar signs and lesions, *Ornithobacterium rhinotracheale* infections and mycoplasmosis may need to be ruled out in turkeys and chickens suspected of having chlamydiosis. This can be accomplished by culturing and serologic testing. Colibacillosis may mimic chlamydiosis to some extent, but it can be excluded by the use of appropriate coliform culturing procedures. Avian influenza virus, paramyxoviruses, herpes viruses or aMPV may have to be ruled out in suspected chlamydiosis by virus isolation and serologic testing.

Intervention Strategies

Management Procedures

Ideally birds should be reared in confinement without contact with contaminated equipment or premises. Contact with potential reservoirs or vectors such as pet birds, rodents, arthropods, and wild and feral birds also should be prevented. General sanitation must be practiced diligently. Movement of people should be restricted so that visitors do

not have free access to premises holding birds. This is easier to accomplish if birds are confined in houses and if the "all-in-all-out" principle is used on the farm.

A sensitive technique for *Chlamydia psittaci* bioaerosol monitoring is available. The air collection medium (ChlamyTrap) used can be examined by PCR or culture. The technique could be used for monitoring the infection pressure in the poultry industries (101).

Susceptibility to Chemical and Physical Agents

The survival of microorganisms in aerosols depends on five different factors: relative humidity (RH), temperature, level of oxygen, presence of UV radiation, and constituents of the aerosol and of air (95). The degree to which these factors influence the survival of microorganisms in aerosols depends strongly on the type of microorganism and the time it has to spend in the aerosol. In general, the following rule applies: Gram-negative bacteria survive best at low temperatures and RH. Thus, *C. psittaci* remains viable at low temperatures and is resistant to desiccation. The bacterium is highly susceptible to repeated freeze-thawing cycles and is destroyed within three minutes when exposed to UV-light.

Chlamydiae are highly susceptible to chemicals that affect their lipid content or the integrity of their cell walls. Even in a milieu of tissue debris they are inactivated rapidly by surface-active compounds, such as quaternary ammonium compounds and lipid solvents (104). Infectivity is destroyed within minutes by exposure to common disinfectants such as benzalkonium chloride, alcoholic iodine solution, 70% ethanol, 3% hydrogen peroxide, and silver nitrate, but they are resistant to cresol compounds and lime. Dilute suspensions (20%) of infectious tissue homogenates are inactivated by incubation for 5 minutes at 56°C, 48 hours at 37°C, 12 days at 22°C, and 50 days at 4°C (88).

Vaccination

Commercial chlamydia vaccines for poultry are not available. Protective immunity to *Chlamydiaceae* is believed to be effected primarily through the action of $CD4^+$ T helper type 1 (Th1) lymphocytes, $CD8^+$ T lymphocytes, mononuclear phagocytes, and cytokines secreted by these cells. In addition, the role of antibodies is not to be underestimated. A protective chlamydial antigen that has been unambiguously identified is the major outer membrane protein (MOMP).

This paragraph summarizes knowledge on *C. psittaci* vaccination experiments in poultry performed during the last decade. In light of current knowledge on protective chlamydial immunity, plasmid DNA expressing the MOMP of *C. psittaci* has been tested for its ability to raise a protective immune response in SPF turkeys against challenge with *C. psittaci* strains (121). Effective priming of T cell memory and significant reduction in clinical signs, lesions, bacterial excretion, and *C. psittaci* replication in tissues was observed. Zhou et al., (138) used a human adenovirus serotype 5 (AdEasyTM-1), which was rendered replication defective by the deletion of the E1 and E3 genes, to obtain a recombinant adenovirus containing the MOMP gene (rAd-MOMP) of a Chinese *C. psittaci* strain of chicken origin. Low mean serum antibody responses (determined by indirect haemaglutination assay) and extremely low mean stimulation indexes in the T cell proliferation assay were observed. The vaccine seemed to induce protection in SPF

chickens based on the observed differences in pathology. Unfortunately, chlamydia excretion and replication in tissues was not examined in this study. More recently, Liu et al., (54), evaluated a recombinant HVT-delivered vaccine against *C. psittaci* and Marek's disease, expressing *C. psittaci* PmpD, in SPF chickens. Post challenge with *C. psittaci* CB7 strain, a significant decrease in respiratory distress, lesions and chlamydia load was found in the vaccinated group compared to the non-vaccinated controls.

Prevention by Means of Ovotransferrin

Ovotransferrin (natural anti-microbial protein) was successfully used to reduce clinical signs, lesions, excretion, and chlamydia replication in experimentally infected SPF turkeys (102). Also, *C. psittaci* infection pressure on a turkey broiler farm was significantly reduced by administering ovotransferrin aerosols. Ovotransferrin significantly reduced respiratory disease, mortality, and antibiotic use on the farm (99).

Treatment

Chlamydiosis treatment for poultry has not changed over the years (113). The drug of choice varies from country to country. Among tetracylines, which are the drugs of choice, chlortetracycline and doxycycline are most often used. Enrofloxacin (fluoroquinolone antibiotic) also can be used, although some countries decided to ban the use of this antibiotic in poultry because of the risk that it promotes drug-resistant bacteria that can be harmful to humans.

Turkeys can be treated with chlortetracycline (CTC) at a concentration of 400 g/ton of pelleted feed. Care must be taken so that heat generated during pelleting does not destroy CTC and lower the active concentration below an effective level. The CTC-medicated feed must be given for 2 weeks and then replaced by nonmedicated feed for 2 days prior to the birds being slaughtered for human consumption. Calcium supplements should not be added to CTC-medicated pellets because calcium ions chelate CTC and diminish its effectiveness. Turkeys also can be treated with doxycycline hyclate formulations for drinking water (doxycycline hyclate 500 mg/g, dose of 20 mg/kg bodyweight/day) for 5 days or enrofloxacin (100 mg/ml) at a daily dose of 10 mg of enrofloxacin/kg of bodyweight, in water, for 3–5 days and in case of mixed or chronic infections for 5–10 days. Medicated drinking water should be replaced every 24 hours. It is recommended that all turkeys on the infected premises be treated.

Essentially the same treatment methods are used to treat other fowl infected with *C. psittaci*. In other birds, salmonellosis may often be a complicating factor so it may be necessary to use a combination of antibiotics. Treatment may not be effective in eliminating the carrier state. Additional periods of treatment may be needed, especially when birds are kept for several weeks.

State Regulations

Because regulations may vary from country to country, the appropriate public health and/or animal health agencies should be consulted as necessary. In many countries, psittacosis (humans) and even chlamydiosis in poultry are notifiable diseases and must be reported within 48 hours.

According to United States Department of Agriculture (USDA) regulations, movement of poultry, carcasses, or offal from any premise is prohibited where the existence of chlamydiosis has been confirmed by isolation of a chlamydial agent. The Animal and Plant Health Inspection Service (APHIS) of the USDA and the U.S. Department of Health and Human Services (US HHS) forbid interstate movement of birds from infected flocks, but there is no restriction on movement of eggs from such flocks, which is not wise considering the possibility of vertical and horizontal transmission of *C. psittaci* through eggs. The European legislation is similar and also rather vague on movement of eggs when *C. psittaci* is suspected or even proven. It states that the competent authority shall ensure that the measures necessary to avoid any spread of disease are taken, in accordance with the requirements of Union legislation governing measures to be taken against the disease in question and on trade in animals.

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