Chlamydophila psittaci: Notes on Some Aspects of Infection, Diagnosis and Treatment

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Introduction

The order *Chlamydiales* contains obligate intracellular parasites that infect and cause disease in a large number of organisms. Within the last 10 years, there has been restructuring of previous taxonomic classifications, due mainly to increasing understanding of molecular and genetic relationships within the order (Bush and Everett, 2001; Everett et al, 1999). Additionally, there have been new species discovered from different ecological niches (Chua et al, 2005; Horn et al, 2000). Figure 1 summarises the current state of classification, while Table 1 highlights the diseases caused by different species of the family *Chlamydiaceae*.

Within avian medicine, *Chlamydophila psittaci* is an important pathogen. It is responsible for clinical disease in avian patients, as well as being present in clinically asymptomatic birds. It is also a significant zoonosis. The challenge of avian medicine is to correlate the available diagnostic tests (all of which have some flaws as screening tests) with the identification of this organism in distinct clinical groups – clinically ill, asymptomatic, exposed and non-exposed birds. Timeliness and accuracy are integral to this diagnostic process.

There are many superb and extensive reviews of *C. psittaci* so this report will not aim to repeat these (Vanrompay, 2000; Vanrompay et al 1995; Andersen and Vanrompay , 2000). Rather, the aim of this report is to highlight particular aspects of this disease and its diagnosis in avian species that may be relevant to the avian practitioner. To do this, occasionally information concerning other species of the *Chlamydiaceae* is mentioned, to allow comparison of chlamydial diseases between species, and to highlight particular features of chlamydial infection.

Life Cycle of the *Chlamydiaceae*

The life cycle is biphasic, with an infectious, metabolically inert elementary body (EB) and a metabolically active, non-infectious reticulate body (RB). The EB is the infectious form of the organism, and is relatively resistant to environmental inactivation (Beatty et al, 1994). Some important constituents of the cell wall are the major outer membrane proteins (MOMP), as well as chlamydial lipopolysaccharide (LPS) (Vanrompay 2000). The MOMP is cysteine rich, and is important in recognition of genus, species and serovar specificity (Vanrompay et al, 1995). Disulfide cross-linking across the MOMP provides additional stability to the EB.

For *C. psittaci,* the infection route is generally by ingestion or inhalation. EBs enter host cells by a process similar to receptor-mediated endocytosis (Gerlach 1994). The intracellular fate of chlamydial organisms depends on type of host cell invaded; chlamydial species or biovar; and state of the chlamydiae at time of uptake into the cell (Darville, 2000).

In normal, productive infections, the EB is contained within a phagosome once inside the host cell. Phagosomes would normally fuse with lysosomes with the host cell cytoplasm, but chlamydial surface antigens inhibit this fusion. This permits chlamydial survival within the cell. Non-viable organisms or those with damaged surface antigens are rapidly destroyed (Beatty et al, 1994).

Within the phagosome, the disulfide bonds of the EB break, and EBs differentiates into RBs. This is the larger, metabolically active form of the organism, but is more fragile than the EB and cannot survive outside the cell (Beatty et al, 1994). RBs undergo a period of growth and binary fission within the phagosome, nourished by surface projections into the host cell cytoplasm (Gerlach, 1994). Once multiplication is complete, the RBs redifferentiate through the intermediate form back into EBs, which are released either by host cell lysis or exocytosis, thus completing the life cycle (Beatty et al, 1994). In cell culture, the length of the developmental cycle is 48-72 hours, dependent upon infecting strain, host cell and environmental conditions (Beatty et al, 1994).

There is substantial evidence that the many of chlamydial species can persist within host cells in a metabolically altered state. Beatty et al (1994) define persistence as a long-term association between chlamydiae and their host cell in which these organisms remain in a viable but culture-negative state. Furthermore, Beatty et al go on to state "although persistence describes a long-lasting association that may not necessarily manifest as clinically recognizable disease, it is distinct from inapparent infections, which may or may not involve evident chlamydial growth." Darville (2000) cites several studies that show that although culture negative, persistent chlamydial infections can be metabolically active. Persistent infections are characterized by a low number of inclusions; differentially regulated synthesis of key chlamydial antigens; the presence of short-lived metabolic products; and retention of viability and metabolic activity (Reveneau et al 2005). In vitro, persistence can be induced with specific antibiotics, specific nutrient deficiencies, cytokines and infection with bacteriophages. Additionally, in some species, monocytes are more likely than other cells to become persistently infected (Hogan et al, 2004). Generally speaking, chlamydial biovars with greatest likelihood of persistence tend to be associated with more widespread, invasive and chronic diseases (Darville, 2000).

Up until the advent of molecular typing, it was difficult to determine if repeated chlamydial presence in an individual was due to persistent or repeated infection. In humans, both persistent and recurrent infections have been demonstrated (Darville, 2000; Joyner et al, 2002; Parks et al, 1997). Infections with *C. trachomatis* in humans can be spontaneously cleared by a percentage of patients, although without treatment, the majority of infections tend to persist (Parks et al, 1997; Joyner et al, 2002).

Pathogenesis of *C. psittaci*

Transmission is generally via aerosol, although ingestion of organisms can result in infection. Vertical transmission has been described in some avian species (Vanrompay et al, 1995).

From a pathogenesis viewpoint, Vanrompay et al (1995) explored the progression of infection of *C. psittaci* in turkeys. In birds infected via aerosol, the epithelial cells of the upper respiratory tract were the initial sites of replication, followed by epithelial cells and macrophages of the lower respiratory tract. Subsequently, there was chlamydaemia, with organisms demonstrated within monocytes and plasma. Organisms then began to appear in epithelial cells and macrophages throughout the body, and were shed in the faeces, from the respiratory tract and in the conjunctiva. Interestingly, this study reported protracted chlamydial excretion from the lateral nasal glands.

The factors that determine spontaneous clearance of infection, versus chronic or persistent infection, are not currently well known. Hogan et al (1994) reviewed the molecular basis for chlamydial persistence.

Clinical Signs

Depending on the virulence of the infecting strain of *C. psittaci* and the species of host, clinical signs in avian patients may range from none through to death. Kaleta (as quoted by Vanrompay 2000) describes five stages of chlamydial infection in psittacine birds, being acute lethal, subacute, chronic, activated latent and subclinical latent forms (Kaleta 1997). All save the latter incorporate anorexia, depression, dyspnoea and diarrhoea as their major clinical signs, none of which are pathognomonic for chlamydial infection (Vanrompay, 2000).

The stereotypical signs of chlamydial disease in birds include respiratory compromise, mucopurulent nasal discharge, diarrhoea, polyuria and lethargy. Often biliverdinuria is present (Vanrompay et al 1995). Neurological signs can be present. In many smaller psittacine species, the clinical signs can be restricted to sinusitis, upper respiratory tract or ocular manifestations.

Respiratory signs are the most common presenting signs in turkeys and ducks, although ducks can develop neurological signs also (Vanrompay 1995). Pigeons can manifest respiratory signs, or a combination of lameness and neurologic signs (Vanrompay 1995).

On gross necropsy, typical lesions include hepatomegaly with miliary necrosis, splenomegaly, fibrinous peritonitis, air sacculitis, perihepatitis, pericarditis, bronchopneumonia, enteritis and nephrosis (Gerlach 1994).

Diagnosis

There is no perfect tool for the diagnosis of *C. psittaci* infections in the avian patient. Each diagnostic modality has advantages and disadvantages. It is up to each veterinarian to evaluate the benefits and consequences of each diagnostic test, and determine which test is applicable in each situation. Table 2 lists commonly available tests within Australia.

Direct visualisation of chlamydial organisms uses specific stains on samples from appropriate areas to diagnose active infection. It requires the harvesting of adequate and appropriate samples, proper staining techniques, and a certain amount of skill in interpretation. It is not a sensitive or specific, but is rapid and inexpensive. Vanrompay (2000) recommends it only for necropsy.

Isolation of *C. psittaci* has been considered the gold standard in diagnosis. The advent of DNA based testing may supercede this. Isolation may give false negatives due to intermittent shedding of the organism, interfering factors on culture, pretreatment of the patient with antibiotics or loss of organism viability during storage and transport (Vanrompay 2000). In the clinical veterinary setting, it is rarely used.

Specific chlamydial antigen detection can detect both viable and non-viable organisms. Most tests use monoclonal antibodies against LPS or MOMP. Vanrompay (2000) states that antigen detection tests are not recommended for use in individual live birds due to sensitivity and specificity issues.

DNA-based testing, which clinically mainly involves PCR assays, is very specific and very sensitive. It has simple transport requirements, but is prone to sample contamination (Vanrompay 2000). PCR will pick up persistent infections, as chlamydial DNA should still be present within the cells (Darville 2000). Veterinarians need to be certain that they are sampling appropriate sites. Vanrompay (2000) strongly states that the respiratory tract is the best sampling site for avian psittacosis diagnosis, due to more marked, and prolonged chlamydial replication in that area. Therefore choanal, or pharyngeal swabs are the most likely to give reliable results for isolation or PCR. Practitioners also need to familiarise themselves with their chosen laboratory's quality control to ensure reliable results.

Serological tests for *C. psittaci* include ELISA, latex agglutination (LA), elementary body agglutination (EBA)

and direct complement fixation (DCF). The EBA detects only IgM, so is useful in documenting early infection. Other tests detect a combination of IgG and IgM, or simply IgM. There can be a high prevalence of anti-chlamydial antibodies in the general avian population (Vanrompay 2000). This makes discrimination between previous infection and spontaneous clearance, previous infection and successful treatment, chronic infections and persistent infections difficult. Additionally, some species do not appear to produce measurable immunoglobulins as effectively as other species (Grimes et al 1996).

In sheep and humans, serology is often used as a flock screening tool, but is not often used for individual animal diagnosis (Papp and Schewen, 1997; Jaeger et al, 2007; Entrican et al, 2001; Johnson et al 2002). In fact, Vanrompay (2000) recommends that serology only be used in conjunction with paired titres, or a direct identification method for the determination of an active infection, as compared to a previous exposure or resolved infection.

Despite these drawbacks, a number of avian practitioners are using serology as a screening tool for avian chlamydial infection. In fact, the American National Association of State Public Health Veterinarians has listed the determination of single high serologic titres against *C. psittaci* as ground for classification of probable (in conjunction with clinical signs) and possible (when asymptomatic) cases of avian chlamydial infections. It is worthwhile noting that the serologic tests currently accepted by this organisation are elementary body agglutination, immunofluorescent antibodies and complement fixation. ELISA serology is not currently accepted by this organisation. This document is reproduced below, and can be found at the following web site: http://www.nasphv.org/Documents/Psittacosis.pdf. It was accessed on July 4th, 2008. Both the American Veterinary Medical Association and the Association of Avian Veterinarians have endorsed this document. It gives the following as criteria for the diagnosis of avian psittacosis:

"A **confirmed** case of avian chlamydial infection is defined on the basis of one of the following:

1. isolation of *C psittaci* from a clinical specimen,
2. identification of chlamydial antigen by use of immunofluorescence (fluorescent antibody) of the bird's tissues,
3. a $\geq$ 4-fold change in serologic titer in 2 specimens from the bird obtained at least 2 weeks apart and assayed simultaneously at the same laboratory,
4. identification of *Chlamydiaceae* within macrophages in smears or tissues stained with Gimenez or Macchiavello's stain. Clinical signs may not be evident.

A **probable** case of avian chlamydial infection is defined as compatible illness and one of the following:

1. a single high serologic titer in one or more specimens obtained after the onset of signs
2. Chlamydiaceae antigen (identified by use of ELISA, PCR, or fluorescent antibody) in feces, a cloacal swab specimen, or respiratory tract or ocular exudates.

A **suspected** case of avian chlamydial infection is defined as:

1. a compatible illness that is not laboratory confirmed but is epidemiologically linked to a confirmed case in a human or bird,
2. an asymptomatic bird with a single high serologic titer or detection of chlamydial antigen,
3. compatible illness with positive results from a nonstandardized test or a new investigational test, or
4. compatible illness that is responsive to appropriate therapy."

It would seem appropriate to include the classification of the diagnosis in the patient's clinical notes (to give an idea of the strength of the practitioner's index of suspicion).

Treatment

Most avian veterinarians will treat with doxycycline, for a 42-45 day period. Treatment can be conducted with injections, with in water medication, or with twice daily administration of oral medication for the required time.
Recently, Reveneau et al (2005) examined both doxycycline and azithromycin activity against *C. trachomatis* in vitro and found that doxycycline was the superior antibiotic against acute infection, whereas azithromycin was superior against persistent infection. Some practitioners have used azithromycin in the treatment of *C. psittaci* in avian species. This appears to have fallen somewhat out of favour, but Reveneau's paper provides some interesting food for thought. Obviously, more research (including pharmocokinetic studies) is required before the standard recommendation for treatment of this disease is altered.

Conclusion

This paper has aimed to stimulate discussion, and highlight some of the considerations involved in treating and diagnosing avian chlamydial infections. This is a common disease in avian practice, and the pathogen has zoonotic implications. However, for all of the prevalence of the pathogen, there are many different schools of thought as to which tests are the most valuable, which screening protocols are more justified and which treatment modality is preferable. Hopefully, these types of discussions will prompt more research in this important field.

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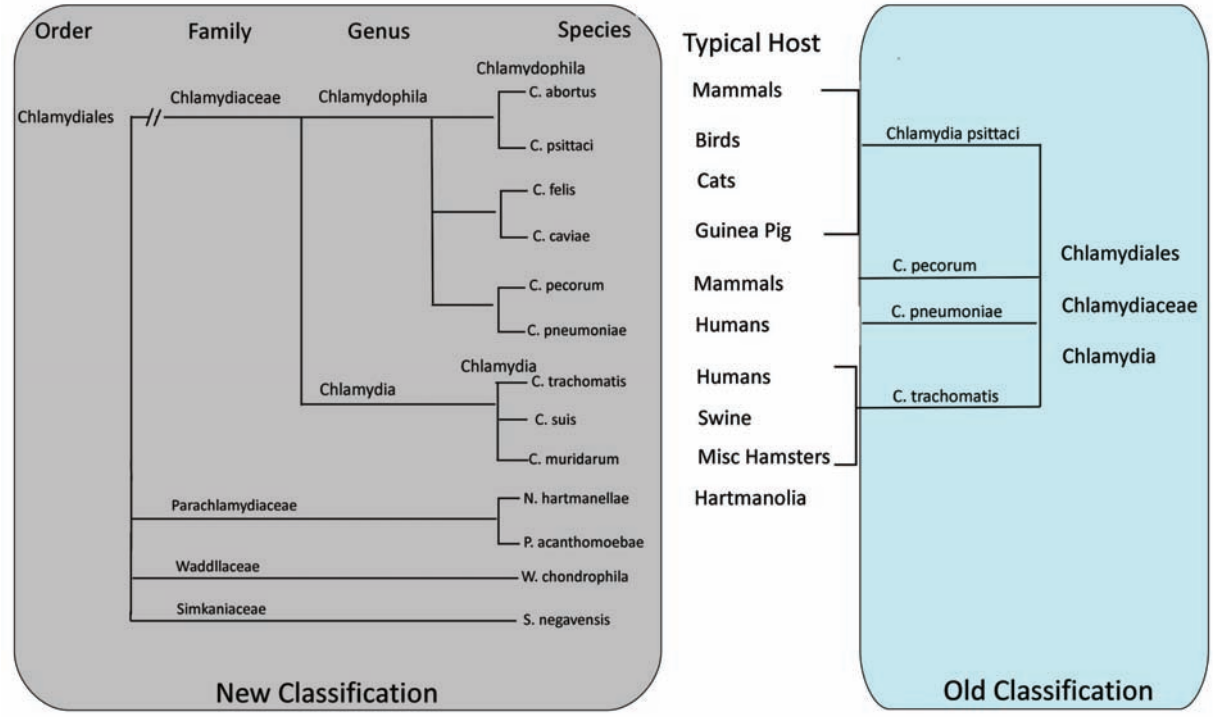

Figure 1: Taken from (http://www.chlamydiae.com/docs/Chlamydiales/diagram/taxondiag.htm - (accessed on July 4th, 2008) and adapted from Bush and Everett (2001)

	Chlamydia			*Chlamydophila*					
	trachomatis	***suis***	***muridarum***	***psittaci***	***pneumoniae***	***pecorum***	***felis***	***caviae***	***abortus***
Route of entry	Pharynx, eye, genital, rectal	Pharynx	Pharynx, genital	Pharynx, eye, genital	Pharynx, eye	Oral	Pharynx, eye, genital	Pharynx, eye, genital, urethral	Oral, genital
Disseminated in blood stream	Yes			Yes	Yes	Yes	Yes		Yes
Asymptomatic persistence in host	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes
Typical host	Human	Swine	Mice, hamsters	Birds*	Humans, koalas, horses	Cattle, sheep, goats, pigs, koalas	House cats*	Guinea pigs	Sheep, mammals*
Infected tissues	Conjunctiva, genital, joints, neonate lung, prostate	Conjunctiva, intestine, lung	Genital, intestine, liver, lung, kidney, spleen	Brain, conjunctiva, genital, intestine, liver, lung, spleen	Arteries, brain, joints, lung	Bladder, brain, conjunctiva, intestine, lymph, joints, prostate	Conjunctiva, genital, joints, lung	Bladder, conjunctiva, genital, lung	Intestine, placenta, spleen, foetal liver
Clinical signs	Conjunctivitis, keratitis, corneal scarring, urethritis, prostatitis, epididymitis, proctitis, pelvic inflammatory disease, tubal factor infertility in women, reactive arthritis	Diarrhoea, enteritis, mastitis, metritis, agalactia, pneumonia, conjunctivitis, pericarditis (NB high prevalence of mixed infections, with *C. abortus*)	Experimental similarity to *C. trachomatis*	Birds - hepatitis, respiratory infection, enteritis; humans - myalgia, atypical pneumonia, fever; dogs - keratoconjunctivitis, respiratory distress, reduced litter size	pneumonia, pharyngitis and bronchitis in humans implicated in atherosclerosis, asthma and chronic obstructive pulmonary disease, poss Alzheimer's as well	Pathogenically diverse, *C. pecorum* strains are associated with pneumonia, polyarthritis, conjunctivitis, abortion, encephalomyelitis, enteritis and diarrhoea	Conjunctivitis, keratitis, gastritis (mild), possibly other syndromes	Conjunctivitis and keratitis in 4-8 week pigs	Abortion, weak neonates Majority ewes uneventful recoveries Cattle disease more episodic Seminitis and vesiculitis in bulls
Diagnosis	Ocular infection - immunofluorescent monoclonal antibody or PCR Genital infection - PCR on first catch urine in men; PCR on tampon or first catch urine or staining techniques or PCR on cervical smear in women	**PCR recommended**		**See notes**	**Varies according to** species	Serology has problems with cross-reaction, and identification of particular genus as multiple species can infect single animal. DNA based techniques advised to allow clear distinction between *C. pecorum, C. abortus* and others	**PCR most** commonly used	Experimentally, use immuno-histochemistry, PCR, histology	Serology - most used for flock diagnosis, and to confirm SPF Latently infected ewes cannot be detected, even by serology More likely to have results with PCR for individual animal diagnosis
Zoonosis				Yes	Yes		Yes	Possible	
References:	Darville,2000; Johnson et al 2002	Pospiscil & Wood, 1987; Reinhold et al 2008; Sasche et al 2003	Imtiaz et al 2006	Arizmendi et al 1992; Sprague et al 2008;	Darville 2000; Jones 1999; Everett et al 1999	Nietfield 2001; Jones 1999	Sykes 2005	Lutz-Wohlgroth et al 2006	Papp and Schewen 1997; Jaeger et al 2007; Entrican et al 2001

Table 1. Adapted from Bush and Everett (2001). * denotes strains often found in alternate hosts

Test	Direct visualisation via cytology	Isolation	Specific Antigen Detection	DNA based tests	Serology
Types of test	Gimenez, Machiavello's, Stamp's	Culture	Direct immunofluorescence, ELISA	PCR tests	Immunocomb (in house ELISA), elementary body agglutination (EBA), latex agglutination (LA), direct complement fixation (DEF), indirect immunofluorescence (IIF) (all external)
Ease	Need training to ID	Difficult. Only detect viable organisms	In house – fussy but not complicated. May need external lab.	Need external lab	Immunocomb fussy, but not complicated. Others external
Cost	Cheap	Expensive	Cheap to intermediate	Cheap to intermediate	Varies depending on test
Equipment	Special stains	Specialised laboratory	Can be done in house	External laboratory	Depends on test
Test Availability in Aus	Yes	Not clinically useful	Clearview Chlamydia Test kits	Yes - several laboratories. Laboratories may vary with respect to timeliness, techniques, quality control	Immunocomb readily available. EBA not commercially available. Laboratories doing DCF, IIF.

Table 2. Diagnostic tests for C psittaci