www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Avian chlamydiosis (psittacosis / ornithosis): diagnosis, prevention and control, and its zoonotic concerns

Kuldeep Dhama[1*], Sandip Chakraborty[2], Ruchi Tiwari[3] and Shambhu Dayal Singh[4]

[1,4]Avian Diseases Section, Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly (UP) – 243122; [2]Animal Resources Development Department, Pt. Nehru Complex, Agartala, Tripura – 799006; [3]Department of Veterinary Microbiology and Immunology, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwa Vidhyalaya EVam Go-Anusandhan Sansthan (DUVASU), Mathura (U.P.) – 281001

Abstract

Chlamydiosis is a contagious disease of pet birds and poultry, having zoonotic implications caused by a bacterium *Chlamydophila psittaci*. In domestic and pet birds, *Chlamydophila psittaci* causes chlamydiosis often referred to as psittacosis or ornithosis or Parrot fever having a significant public health impact. A special feature of *Chlamydophila* is that it has a biphasic life cycle existing as elementary, reticulate and intermediate bodies. Young birds are generally more susceptible. The organisms are shed in the nasal and ocular secretions. Fecal material or feather dust is resistant to drying and can act as source of infection. Vertical transmission through eggs has been described for ducks and chickens; turkeys and a number of wild birds. Pneumonia is a constant feature and lesions involve multiple organs. A short lived immunity to infection develops. The major outer membrane protein (MOMP) is immunodominant in nature and has a protective role in immunity. Usual diagnosis is based on the isolation of the organism in chicken embryo and cell lines and staining with special stains like Gimenez; Castaneda or Macchiavello's. A wide variety of serological techniques including enzyme linked immunosorbent assay (ELISA); immunofluorescence and immunoperoxidase; agglutination tests are available. The advent of molecular techniques including polymerase chain reaction (PCR); restriction fragment length polymorphism and deoxyribonucleic acid (DNA) sequencing has greatly aided in the diagnosis. Lipopolysaccharide (LPS) and major outer membrane protein (MOMP); OmpA; *pmp*-gene and Dna-K like protein are the main targets for serological as well as molecular detection techniques. Psittacosis in human is a disease of increasing concern and occurs in both sporadic as well as epidemic forms. Psittacines, pigeons and turkeys mainly transmit the disease. Elementary bodies (EB) are major source of human infection. Inactivated vaccines are used generally as there are chances of carrier infection with live vaccines and require multiple administration. Recently, DNA vaccines and ovotransferrin therapy have gained popularity. Strict hygiene and sanitation along with public awareness are essential to prevent the disease. The present review describes the avian chlamydiosis in detail focusing on the etiological agent, the disease and its epidemiology, and the trends in diagnosis, prevention, treatment and control along with its public health concerns.

Keywords: Avian chlamydiosis; psittacosis; *Chlamydophila psittaci*; poultry, zoonosis.

To cite this article: Dhama K, S Chakraborty, R Tiwari and SD Singh, 2013. Avian chlamydiosis (psittacosis / ornithosis): diagnosis, prevention and control, and its zoonotic concerns. Res. Opin. Anim. Vet. Sci., 3(6), 157-169.

Introduction

Chlamydiosis is a contagious disease of pet birds and poultry, having zoonotic implications, caused by a bacterium *Chlamydophila psittaci* (Harris, 1983; Vanrompay et al., 1995; Chandra et al., 2001; Andersen and Vanrampay, 2003; Dhama et al., 2008a). It is listed disease by World Organization for Animal Health / Organization International des Epizooties (OIE) and about 465 avian species are affected by infection due to this organism worldwide (Andersen, 2000; Dhama et al., 2011; OIE, 2012). In domestic and pet birds,

Corresponding author: Kuldeep Dhama, Principal Scientist, Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.) – 243122, India.

Chlamydophila psittaci causes chlamydiosis often referred to as psittacosis or ornithosis or Parrot fever having a significant zoonotic impact (Woldehiwet, 2001; Andersen and Vanrompay, 2003). All avian species are potential hosts for *Chlamydophila* (*C. psittaci* isolated from more than 140 avian species) but the disease more commonly affects pet birds like parakeets and budgerigars (Brand, 1989; Chandra et al., 2001; Andersen and Vanrampay, 2003). As far as the poultry industry is concerned, turkeys and ducks are susceptible to chlamydiosis, while chickens are relatively resistant and thus are rarely affected. *C. psittaci* is endemic worldwide and is well adapted to avian hosts, but only occasional epidemics occur in poultry. Clinical disease is often seen as a result of poor husbandry practices, overcrowding or malnutrition. The organism produces a systemic and occasionally fatal disease in birds which is often transmitted by the inhalation or ingestion of infectious fecal dust. The disease in pet birds and poultry are quite similar. Morbidity and mortality vary with the host species and pathogenic potential of the bacterial serotype. Affected birds whether domestic or pet ones, generally experience anorexia, depression, nasal and ocular discharge along with respiratory distress. Avian chlamydiosis can occur in six major domestic species (chicken, turkey, Pekin duck, Muscovy duck, goose, and pigeon), three minor domestic species (Japanese quail, bobwhite quail, and peafowl) and a total of 460 free-living or pet bird species (Andersen and Vanrompay, 2003; Kaleta and Taday, 2003; Dhama et al., 2008a). Concurrent infections or stress increases the severity of the disease. Avian chlamydiosis is also considered as a major zoonotic disease that affects humans who are involved in poultry and pet bird rearing. Human disease can occur even if there is only brief proximity to a single infected bird. The disease gained worldwide prominence during a pandemic in 1929-1930 involving 12 countries, after which only occasional outbreaks occurred in poultry flocks which lead to fewer zoonotic incidences. Predominantly, the pet birds have served as a source of infection to humans. Infected birds shed the bacteria through feces and nasal discharges, and humans become infected from exposure to these materials which can cause severe pneumonia and other serious health problems (Andersen and Vanrompay, 2003; Smith et al., 2005). Recent advances in diagnosis and control are playing crucial role in prevention, controlling and treatment of avian chlamydiosis and reducing the zoonotic threats (Kataria et al., 2005; Dhama and Mahendran 2008a; Dhama et al., 2008b, 2011, 2012). The present review describes the avian chlamydiosis in detail and the ongoing trends in its diagnosis, prevention, treatment and control along with emphasis on zoonotic significance of the disease.

The Etiological agent

Chlamydophila psittaci is an obligate intracellular gram-negative coccoid bacteria. The family Chlamydiaceae was recently reclassified into two genera and nine species. The genus *Chlamydia* having three species viz. *C. trachomatis* (human beings), *C. suis* (pigs), and *C. muridarum* (rodents); and the genus *Chlamydophila* having six species viz. *C. psittaci* (birds), *C. felis* (cat), *C. abortus* (cattle, sheep and goat), *C. caviae* (guinea pig), *C. pecorum* (sheep and cattle) and *C. pneumonia* (human beings) (Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). Within *Chlamydophila psittaci*, there are currently six serotypes (A to F) that infect birds, and which shows host specificity: A and F- for parrots, parakeets and budgerigars; B-pigeons; C-ducks and geese (water fowl); D-turkeys; and E- pigeons, ducks, ostriches and rheas. These serotypes are distinct from those associated with chlamydiosis in mammals. Apart from six known avian serovars, two mammalian serovars, M56 from muskrats and WC from cattle also exist. Serotype D usually cause severe disease in poultry and is also having high zoonotic potential, thus the veterinarians and poultry workers are especially at risk of becoming infected by D strains. Serovar B strains are less pathogenic to humans in comparison with A strains (Everett et al., 1999; Meijer and Ossewaarde, 2002). In turkeys, about 50-80% of the birds may show clinical signs with a mortality of 10-30%. In broiler birds, the mortality can go to as high as 80%. Other serotypes, irrespective of being either pet birds or poultry, are reported to be causing lesser mortality (Vanrompay et al., 1995). Chlamydial strains from mammals are not a problem for poultry. Experimental hosts of avian chlamydiae can be any species of birds, but species vary in susceptibility; mammalian laboratory hosts being mice and occasionally guinea pigs (Woldehiweit, 2001).

A special feature of *Chlamydophila* is that it has a biphasic life cycle, existing as elementary, reticulate and intermediate bodies. Elementary bodies (EB) are infectious particles that exist outside the host whereas reticulate bodies (RB), formed from EB, are metabolically active particles that replicate by binary fission within host epithelial cells and are larger in size (Andersen and Vanrompay, 2003; Horn et al., 2004). Later, these reticulate bodies condense to form elementary bodies and are released from host cells to the environment. During this maturation, morphologically intermediate forms (IB) can be observed inside the host cells. EB are highly resistant to the hostile extracellular environment; found in feather dust, feces, urine, and ocular, nasal and respiratory secretions may infect healthy birds, attaches to the target epithelial cells and gains entry (Moulder, 1991; Horn and Wagner, 2001). Within the carcass these may

not survive putrefaction. Persistent infections in carrier birds can be latent and during a stressful situation, it could lead to the emergence of clinical disease and shedding of the organism to environment (Hogan et al., 2004). As complete genome sequences and advanced cell biology techniques are available it has become easier nowadays to understand the developmental cycle of the organism by measurement of the transcriptional changes throughout the replication cycle (AbdelRahman and Belland, 2005).

Epidemiology, transmission and spread

C. psittaci is found worldwide and especially in tropical and subtropical countries the organism is commonly distributed (Harkinezhad et al., 2009). Wild and feral birds are the common reservoirs of the organism that intermingle with domestic birds freely (Chahota et al., 2006). The organisms shed in the nasal and ocular secretions, fecal material or feather dust are resistant to drying and can remain viable and infectious for several months outside the host, which when inhaled by susceptible birds, causes the disease. The elementary body (EB) can survive in dried feces for months and plays vital role in dissemination of the disease in flocks. The time between exposures to the onset of infection (incubation period) ranges from 3 days to several (2-8) weeks. However, in latent infections, active disease may be seen years after infection. Generally, the infection is transmitted by aerosols and contaminated dust, and the birds are commonly infected when aerosolized EB are ingested or inhaled. After entry into the body, mainly by inhalation, the organisms multiply in the lungs, air sacs and pericardium and by hematogenous route it spreads to liver, spleen and kidneys where further replication occurs, and RB and EB are produced (Chandra et al., 2001; Andersen and Vanrampay, 2003; Dhama et al., 2008a). The sources of infection include birds in the incubative stage of infection, sick birds, carriers and infected inanimate objects. Biting insects, mites, and lice may play a vital role especially in mechanical transmission of the organism. Infected birds can also remain as asymptomatic carriers and shed *C. psittaci* intermittently, during stressful times. The disease has a greater chance of spreading and/or precipitating in overcrowded conditions; stale air environments, nest-boxes, and brooders; shipping/movement stress; change of diet or environment; and concurrent infections with other organisms such as salmonellae or *Pasteurella multocida* (Chandra et al., 2001; Andersen and Vanrampay, 2003). Stress factors are known to activate shedding of the chlamydophila to the environment. Vertical transmission through eggs has been described for ducks, chickens, turkeys, and a number of wild birds, however, its occurrence appear to be fairly low. Transmission of the chlamydia from birds to humans has been confirmed in a number of cases (Chandra et al., 2001).

Clinical signs

Young birds are generally more susceptible than older birds to infection, clinical disease and mortality. Clinical signs may be absent, mild or severe depending on the strain of *C. psittaci* and host infected (Vanrompay et al., 1995; Chandra et al., 2001; Andersen and Vanrampay, 2003; Dhama et al., 2008a). There are two strains of *C. psittaci* isolated from the domestic fowl: highly virulent avian strain - causes 5-30% mortality with acute epidemic progression, and low virulence strain - causes slowly progressive epidemics with a mortality of less than 5%. Highly virulent strains, isolated most often from turkeys and occasionally from clinically normal wild birds, are also termed as 'toxigenic', because in natural and experimental hosts they produce fatal disease with lesions characterized by extensive vascular congestion and inflammation of vital organs. Severe damage to the lungs and heart is a major cause of death. Low virulence strains are routinely isolated from pigeons and ducks and occasionally from turkeys, sparrows, and other wild birds. These cause gross lesions, which are similar to those caused by virulent strains, but are less severe and extensive (Tully, 1993; Andersen and Vanrompay, 2003; Dhama et al., 2008a).

Chickens are relatively resistant to disease caused by *C. psittaci*. Acute infection in chickens progresses to disease and mortality only in young birds, and the incidence of actual epidemics is very low. Chlamydiosis can result in serious economic losses in turkey or duck operations. In poultry, the disease predominantly affects air sacs and lungs. Intense replication of the organism occurs in the respiratory tract and septicemia occurs. Egg production may be decreased (10-20%) (Arzey and Arzey, 1990). Some birds die abruptly due to the rupture of liver and spleen (Dorrestein, 2009). Clinical signs in poultry include depression, inappetence, weight loss, cachexia and lethargy; Reduced egg production; low body temperature (hyperthermia); ruffled feathers, rhinitis, sinusitis and dyspnea; nasal and ocular discharges; yellowish-green gelatinous droppings or watery diarrhea; unilateral or bilateral keratoconjunctivitis (Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). In turkeys, orchitis and epididymitis have also been reported and often the immediate cause of death in adult males is due to rupture of testicular blood vessels followed by massive internal bleeding. Occasional nervous signs, tremors and imbalanced gait have been observed in ducks (Newmann, 1989).

In pet birds, common symptoms include anorexia, unthriftiness, weight loss, diarrhea, yellowish droppings, sinusitis, respiratory distress, conjunctivitis, swollen eyelids, and rhinitis. Respiratory difficulty is accompanied by rattling sounds (Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). As disease progresses, birds become weak and emaciated. Some times nervous signs including ataxia, trembling or gait abnormalities are noticed. Asymptomatic infections or mild infections with diarrhea and mild respiratory signs are also observed. Infection in pigeons is endemic and is believed to be perpetuated primarily by a parent-to-nestling transmission cycle. Recovered birds become carriers without signs of disease. In pigeons, salmonellosis or trichomoniasis exacerbates the illness in carrier birds, inducing signs and lesions of acute disease (Harlin and Wade, 2009; Olsen, 2009).

Lesions

Generally, the lesions found during postmortem in birds include pneumonia and congestion of lungs, clouding of air sac walls, airsacculitis, hepatitis, pericarditis, peritonitis, hepatomegaly and splenomegaly (Vanrompay et al., 1995; Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). Serofibrinous exudates are found on all organs and tissues of the thoracic and peritoneal cavities. The heart may be enlarged, and its surface may be covered with thick fibrin plaques or encrusted with yellowish, flaky exudates. Thickened air sacs are heavily coated with fibrinous exudate. Spleen becomes enlarged, dark, and soft and may be covered with grey-white necrotic spots. Necrotic foci and petechiae can be observed in liver. The peritoneal serosa and mesentry show vascular congestion and may be coated with foamy, white fibrinous exudates. In pet birds, tracheitis, airsacculitis, pneumonia and focal necrosis of the liver, are observed. The liver is usually swollen, soft and discolored. The spleen may be enlarged, soft and dark (Van Buuren et al., 1994). In case of turkeys three strains of *Chlamydophila psittaci* viz., TT3, VS1 and B577 cause pericarditis, airsacculitis and bronchopneumonia; and lateral nasal adenitis respectively (Tappe et al., 1989). Concurrent infection with salmonellae is often observed in chlamydiosis in pigeons, ducks and some psittacine birds; which increase the mortality rate in birds, and chlamydiae are shed in very large numbers leading to the exposure of the susceptible hosts in the immediate environment to the doses that result in clinical disease (Andersen, 1998).

Zoonotic significance

Psittacosis in human is a disease of increasing concern and occurs in both sporadic as well as epidemic forms (especially amongst workers in poultry farms having exposure to chickens; ducks and turkeys). The increasing demand for the pet and fancy birds and lack of awareness about the disease has contributed much to the persistence of avian chlamydiosis among the pet birds, poultry as well as human population. Psittacosis can be a serious human health problem, infecting more frequently those who are involved in pet bird or poultry rearing (Harris, 1983; Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a; Dhama et al., 2011). The economic losses and the human infections usually follow a sporadic pattern. Children and old age people gets the infection very easily, which is characterized by severe pneumonia. Between 1988 and 2003, Centers for Disease Control (CDC), Atlanta reported more than 900 human cases in USA (CDC, 1997 and 2003). Mostly, the disease was contracted from psittacines, pigeons, and turkeys. Chickens, pheasants, quail and patridges also may pose public health threats, mainly to their producers (Harkinezhad et al., 2007; Vanrompay et al., 2007). In man, ornithosis is considered as a milder disease than psittacosis, however, the disease in man contracted from turkeys is more severe than from psittacine birds (Van Loock et al., 2005). The disease in ducks, turkeys and feral pigeons is of particular concern as transmission to humans is common during handling, slaughtering and processing of the birds. Human infections usually occur by inhalation of aerosol, as the infected birds shed elementary bodies in their excretions and secretions. Secondary spread among humans rarely occurs (Moroney et al., 1998). The incubation period is 5 to 14 days, however longer periods are also known. The infection in man varies from a mild flu-like infection with fever, shivering, chilling, severe headaches, anorexia, general debilitation, sore throat, malaise, myalgia and photophobia to a serious atypical pneumonia with dry hacking cough, dyspnea and neurological signs, and if not treated the outcome can be fatal. Rarely, severe illness with myocarditis and renal complications may develop. Encephalitis, meningitis, and myelitis have also been reported in human cases (Saito et al., 2005; Heddema et al., 2006a). Affected human beings can be treated with tetracycline or doxycycline for a period of 3 weeks (Stewardson and Grayson, 2010). In human beings, similar to birds, diagnosis requires culture of organism or detection of chlamydial antigen or anti-chlamydial antibodies (Raso et al., 2010). PCR can be used to genotype all *C. psittaci* and can help to understand the prevalence of different genotypes (Heddema et al., 2006b). Timely detection and proper treatment will never make the disease progress to fatal proportions. Moreover, a thorough knowledge of the disease and following the principles of biosecurity can easily thwart the threat which this unique organism poses to poultry as well as human health (Harkinezhad et al., 2009; Dhama et al., 2012).

Immunity

Immunity to Chlamydia is poor and short lived. Older birds show more resistant to clinical disease, even though infection may occur. The major outer membrane protein (MOMP) of *C. psittaci* is an immunodominant protein, and has a protective role in immunity to chlamydial infection. Important host defense mechanisms against *Chlamydia* remain incompletely defined (Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). Varying level of Th1 cell responses govern the progression of the disease as well as control and clearance of the organism. The efficacy of Th1 response that can eliminate the intracellular organism from the host system is reduced if the duration of the lytic cycle extends beyond the normal (Wilson et al., 2003). It has been observed recently that *C. psittaci* limits its replication deliberately and gains the capability to infect other cells after infecting blood monocytes/ macrophages which act as a vehicle for quick transport in the host system (Beeckman and Vanrompay, 2010). Macrophage deactivation can however occur due to excessive production of interleukin – 10 (IL-10) due to concurrent infection with *Escherichia coli* thereby inhibiting the Th1-promoting response which is believed to aggravate the pathological condition in such instances (Beeckman et al., 2010).

Diagnosis

Collection of informations at definite interval after the date of first infection is essential for conducting controlled studies. Usual diagnosis is based on the isolation of organism in cell cultures, staining techniques such as Machiavello or Gimenez and antigen or antibody detection using serological techniques such as AGID, ELISA, FAT and CFT (Trevejo et al., 1999; Elder and Brown, 1999; Woldehiwet, 2001; Raso et al., 2002; Andersen and Vanrompay, 2003; Kaleta and Taday, 2003; Dhama et al., 2008a). Definitive diagnosis of *C. psittaci* is done by isolating and identifying the organism in culture or by demonstrating the chlamydial antigens or genes in clinical samples (Andersen, 1998; Andersen, 2000; Charlton, 2000; Chandra et al., 2001; Andersen and Vanrompay, 2003). Cultural isolation is obviously the gold standard test but to perform it is difficult and requires hard labor. Demonstrating rising antibody titres (four-fold increase) in paired acute and convalescent sera, or a high number of positive titres in a flock is a good indication of *C. psittaci* infection. Recombinant ELISA is an important test to detect *C. psittaci* especially in psittacine birds and with the advent of recombinant DNA technology is easier to perform as well (Vanrompay et al., 2000). A micro-immunofluorescence test (MIFT) was recently used with a panel of serovar-specific MAb's to serotype chlamydial isolates from birds (Andersen, 2005). Currently, the diagnosis of chlamydial infections is essentially based on molecular methods (Andersen and Vanrompay, 2003; Jaton and Greub, 2005). Tissue and fecal specimens of the suspected birds can be tested by PCR and the sensitivity increased by nested PCR (Hewinson et al., 1997; Trevejo et al., 1999; Sachse and Hotzel, 2002). Messmer et al. (1997) have developed a multiplex PCR for the differential detection of Chlamydial species. A *C. psittaci* species-specific real-time PCR targeting the ribosomal spacer and a genotype-specific real-time PCR targeting the outer membrane protein A (ompA) gene was developed which detected genotypes A to F, along with a new E/B genotype (Geens et al., 2005a). In order to increase sensitivity and at the same time to circumvent PCR inhibitors in clinical specimens nested PCR strategy is helpful. Advantage of using such technique is that it helps in determining whether both live as well as dead birds are infected with the organism or not (Messmer et al., 2000). Recently, a DNA microarray assay for detection and identification of chlamydiae was developed (Sachse et al., 2005). As an alternative to isolation of *C. psittaci* by cell culture, a microarray test has also been proposed (Sachse et al., 2009a).

During necropsy, findings such as slightly thickened or cloudy air sacs, hepatomegaly, and splenomegaly are suggestive of chlamydiosis. All chlamydiae are gram-negative, but the gram stain is of no practical value in identifying chlamydiae. Impression smears are made from exudates, lesions or the surface of the liver or spleen, cloacal, tracheal or conjunctival swabs, and may be stained with Giemsa, Gimenez, modified Gimenez (PVK stain), Castaneda, Macchiavello's or Stamp staining methods to identify the organism by light microscopy (Vanrompay et al., 1992; Black, 1997; Dhama et al., 2008a). In wet mounts of impression smears of infected tissues or exudates, intracellular chlamydiae are large enough to be seen by phase contrast microscope. They are also readily seen by dark-field microscopy. Samples should be collected aseptically, as contaminant bacteria can interfere with test results. Tissues viz. air sacs, spleen, pericardium, heart, liver and kidney are the tissues of choice to be collected at postmortem. Oropharyngeal and cloacal swabs need to be collected from live birds (Andersen, 1996).

For routine diagnosis, direct immunofluorescence staining is the test of choice. Similar to this test DNA spot hybridization can detect both viable as well as non-viable organisms (Timms et al., 1988). For isolation of *C. psittaci* from affected birds, processed specimen is inoculated in cell cultures (L, McCoy, HeLa, Vero, L929 and BGM cell lines), embryonated chicken eggs (yolk sac route) or mice (Chandra et al., 2001; Andersen and Vanrompay, 2003). Proper handling of

the samples is necessary to prevent loss of infectivity of chlamydiae during shipment and handling. Samples for bacterial isolation viz. lung, liver, spleen, kidney and intestine, must be collected aseptically and placed in sterile sucrose-phosphate-glutamate (SPG) buffer, a specific transport medium for chlamydia, with 10% serum, and antibiotics which reduce contaminants but have no adverse effect on chlamydiae. The transparent medium can be used as a diluent for freezing of chlamydia. The samples should not be frozen if they can be processed in 2-3 days. Oropharyngeal swabs are more consistent for isolation of the agent than fecal swabs, especially during early stages of infection. For egg inoculation, eggs must be from chickens that are chlamydial free and that are not consuming chlamydiastatic antibiotics in their feed. *C. psittaci* causes vascular congestion in the yolk sac and death of the embryos at 3-10 days after inoculation. One or two blind passages should be performed, if no embryos die (Vanrompay et al., 1992 and 1993). Yolk sac harvest or touch impressions could be subjected to staining protocols for identifying the organism (Wittenbrink et al., 1993). Buffalo green monkey (BGM) cells can also be used for isolation of Chlamydial antigen followed by subsequent indirect immunofluorescence test for the purpose of serotyping for which a panel of monoclonal antibodies is used (Vanrompay et al., 1993). Chlamydial inclusions can be demonstrated in infected cell cultures, embryos and tissues of inoculate mice by cytological staining or immunofluoresence staining using monoclonal antibodies against the genus-specific epitope on the chlamydial LPS or the MOMP (Messmer et al., 2001; Zhou et al., 2007).

Also, demonstrating *C. psittaci* in tissues, feces, or exudates by immunohistochemical staining, fluorescent antibody technique (FAT), antigen capture ELISA, latex agglutination (LA) and elementary body agglutination (EBA) can be performed (Evans et al., 1983; Andersen and Vanrampay, 2003). The sensitivity of LA is higher in comparison to tests like peroxidase-antiperoxidase reaction (PAP) and even cultural isolation (Moore et al., 1991). Immunochromatography (IC) and counter immunoelectrophoresis are also available as a rapid diagnostic test for demonstrating chlamydial antigen (Persson and Boman, 2000). The use of immunohistochemical staining of formalin-fixed sections is gaining popularity (Brown, 1998). Complement fixation test (CFT) is considered as a standard serological assay by OIE, in which the antigen used being a group-reactive lipopolysaccharide (LPS) present in all strains of *Chlamydophila*. Generally, high titres (≥64 in poultry) are indicative of a current or recent infection. Cell culture propagated chlamydiae is used as antigen (Grimes and Arizmendi, 1996). CFT is able to detect organisms in tracheal and cloacal swabs of parrots and macaw when used along with semi-nested PCR (Raso et al., 2006). The species within *Chlamydia* and *Chlamydophila* are distinguished by either serology, restriction fragment length polymorphism (RFLP) or by sequence analysis (Everett and Andersen, 1999). Monoclonal antibodies (MAbs) to LPS play crucial role in detection of chlamydiae. *C. psittaci* serovars can be distinguished by use of a panel of serovar-specific MAbs in micro-immunofluorescence test. Depending on the type of samples gene-based diagnosis proves to be more sensitive than either cultural isolation or immunoassays (Fudge, 1997). Recently, molecular biological techniques like polymerase chain reaction (PCR) especially multiplex-PCR, which targets the MOMP, tRNA-gly, 23S or the 16S ribosomal RNA genes allows rapid identification of the chlamydial species at genomic levels (Fukushi and Hirai, 1989; Hewinson et al., 1997; Messmer et al., 1997; Everett et al., 1999; Dhama et al., 2008a; Dhama et al., 2008b). 16SG: a PCR protocol wherein 16S rRNA gene is targeted by coupling with high resolution melt (HRM) is helpful for detection of *C. psittaci* in chicken (Robertson et al., 2010).

PCR may sometimes reflect a carrier status, in which cases quantitative PCR or serology may be helpful to confirm the clinical diagnosis of chlamydiosis (Takashima et al., 1996; DeGraves et al., 2003). Real-time PCR can detect the *omp*A gene and thus is helpful to differentiate *C. psittaci* from *C. abortus* in tissue samples (Pantchev et al., 2009). Cell cultures can be analysed rapidly and efficiently by flow cytometry and the correlation between the copy numbers of the organism and increase in positive cells with infectious dose can be calculated by using real-time PCR. Advantage is that large number of samples can be handled by use of such technique (Grun et al., 2009). DNA hybridization using a plasmid probe specific for the chlamydial strain of avian species can detect less than 10^5 elementary bodies (Rasmussen and Timms, 1991). RFLP analysis of the *omp*A gene of *C. psittaci* serovars reveals corresponding restriction patterns or genotypes (Geens et al., 2005b). When it is impossible to grow the bacterium from pathological samples RFLP analysis of the *pmp*-gene is found to be helpful (Laroucau et al., 2007). DNA sequencing is the most reliable way to distinguish *Chlamydia* and *Chlamydophila* (Sudler et al., 2004). For differentiation of various *C. psittaci* isolates restriction mapping targeting the *omp*1 gene by native DNA based RFLP and DNA-DNA hybridization is useful (Sayada et al., 1995). Dna-K like protein of *C. psittaci* is an important target for cloning and expression (considered as a superior diagnostic methodology) and results can subsequently be evaluated by combined use of immunobloting and indirect ELISA (Anderson et al., 1997). Measurement of the level of 16S rRNA

transcript combined with microarray technology can help to identify the gene signals triggering initiation of replication of chlamydial body or its transformation to infectious form; thereby determination of chlamydial generation time becomes much accurate (Wilson et al., 2004). The requirement for bacterial growth and development within the host system can further be assessed by temporal gene expression profiling (AbdelRahman and Belland, 2005). In order to achieve high degree of sensitivity as well as rapidity and reproducibility for genotyping of strains of *C. psittaci* array tube assay proves to be suitable (Sachse et al., 2009b). For the purpose of detecting tandem repeats across the genome (whole) of the organism multilocus variable number of tandem repeats (VNTR) analysis (MLVA) system is useful. It provides a higher level of differentiation additionally with identification of distinct band patterns of the selected gene loci and suits well for molecular epidemiological studies (Laroucau et al., 2008).

For a successful differential diagnosis in poultry, one has to rule out influenza, aspergillosis, fowl cholera, mycoplasmosis and colibacillosis. In pet birds, herpes and paramyxovirus infections, influenza, and enterobacterial infections has to be ruled out due to the similarity in symptoms (Andersen and Vanrompay, 2003; Dhama et al., 2008a).

Prevention and control

Regarding the control and preventive measures sought against avian chlamydiosis, the biggest lacuna is the lack of proper immunoprophylactic agents (Andersen, 2000; Chandra et al., 2001; Andersen and Vanrompay, 2003). Chlamydial vaccines are not available commercially. For the effective control of chlamydiosis in birds during a suspected infection, proper disinfection of the poultry environment and treatment of the birds with broad spectrum antibiotics such as chlortetracycline and doxycycline are mandatory (Woldehiwet, 2001; Andersen and Vanrompay, 2003; Jaton and Greub, 2005). For immunoprophylaxis, live and inactivated vaccines (bacterins) are available but inactivated vaccines are used generally as there are chances of carrier infection with live vaccines and requires multiple administration (Woldehiwet, 2001, 2006). There is however need to identify antigens for candidate vaccine that is aided by the representative genome sequencing in addition to which identification of suitable adjuvant is essential to elicit mucosal as well as systemic and humoral immunity. Especially in terms of safety and stability much promise is provided by DNA vaccines (Longbottom and Livingstone, 2006; Dhama et al., 2008c). Current research is on for developing new generation vaccines for prevention of chlamydial infection in birds. A Plasmid DNA expressing the major *Omp* of an avian *Chlamydophila psittaci* serovar A and D strain has been found to induce protective immunity against *C. psittaci* challenge with significant reduction in clinical symptoms (Vanrompay et al., 2001b; Vanloock et al., 2004). In another trial, a significant level of protection was observed in birds immunized with interferon gamma (IFN-gamma) along with MOMP DNA vaccine (Vanrompay et al., 2001a). In turkeys CpG motifs can be used as adjuvant in DNA vaccination (Loots et al., 2006). Recently, Vitamin D (3) was used as an immunomodulant along with a plasmid DNA vaccine, the use of which elicited a good immune response with augmented serum and mucosal antibody titres (Verminnen et al., 2005). DNA immunization by gene gun technique can deliver complementary DNA (cDNA)/ MOMP even in microgram concentration. By this method both T as well as B lymphocytes get primed and in order to prevent severe clinical signs and excretion of the organism this is found to be an effective method (Vanrompay et al., 1999).

Currently, antibiotics are the only means of controlling avian chlamydiosis. Therapy with anti-chlamydial antibiotics such as tetracyclines, chlortetracyclines (CTC), macrolides, erythromycin, fluoroquinolones, and chloramphenicol are found to be effective, but require a long term therapy (about 45 days) to clear the infection (Andersen and Vanrompay, 2003; Dhama et al., 2008a). The lower doses are effective against severe disease but the higher doses are necessary for attempted elimination of the organism. Avoid calcium supplementation while giving CTC medicated pelleted feed since calcium ions chelate CTC and diminishes its effectiveness (Samour, 2000). Penicillin, D-cycloserine and doxycycline are also reported to be effective. Reinfection can occur readily because wild birds may continue to harbor chlamydiae, or the treatment might not make all birds chlamydiae free. Salmonellosis may often be a complicating factor, where combination of drugs is desired. In pigeons, drug treatment may not be effective in eliminating the carrier state. Alternate periods of treatment with periods of no treatment may eventually clear the chronic infection (Gylsdorff, 1987; Flammer et al., 2001). Ovotransferrin (ovoTF) have been found to be much effective in order to prevent bacterial attachment and entry into the host cell followed by actin recruitment reduction at the bacterial entry point in a dose dependent fashion; thereby playing role in prevention of avian chlamydiosis (Caroline et al., 2009).

Human population that comes in contact with dust contaminated with *C. psittaci viz.,* construction workers must be educated well and communicated well regarding the health risk issues. Wearing of protective clothings like hoods, boots and gloves; air filter and face masks at work places are recommended for such

people especially when they come in contact with pigeon excreta as feral pigeons can act as an important transmitter of the disease in human population (Magnino et al., 2009).

Common disinfectants comprising of quarternary ammonium compounds, lipid solvents, benzalkonium chloride, iodophores, alcoholic iodine solution, 70% ethanol, 3% hydrogen peroxide, formaldehyde and silver nitrate are very effective in rapidly destroying the infectivity of the organisms (Chandra et al., 2001; Andersen and Vanrompay, 2003; Dhama et al., 2008a). But chlamydiae are resistant to cresol compounds and lime. They are somewhat less susceptible to ethanol, methanol, zinc sulphate, phenol, hydrochloric acid, or sodium hydroxide. Being a contagious disease, another best option is the implementation of strict biosecurity measures so as to limit the transmission of the agent as well as to lessen its zoonotic potential (Dhama et al., 2008a, 2011, 2012). This includes birds should be reared in confinement without any contact with potentially contaminated premises. Contact with reservoirs or vectors (wild and feral birds) should also be prevented. Visitors should be restricted. Strict trade/import regulations and appropriate biosecurity measures are very essential to check the disease. Movement of poultry, carcasses, or offal from any farm where chlamydiosis has been diagnosed should be prohibited. Proper disposal of dead birds should be followed. The affected birds must be kept in quarantine during treatment. While antibiotic therapy is followed, the premises should be cleaned and disinfected frequently. Attention should be paid to minimize the spread of infected dust. Dry and dusty areas in and around poultry or pet bird premises can be disinfected with household bleach or a commercial disinfectant. General principles of maintenance of strict hygiene and sanitation have to be followed. When collecting samples from dead or live birds, every precaution should be taken by personnel to avoid infection. While handling infected birds, or carcasses being suspected for being infected with *C. psittaci,* contaminated materials or cleaning infected premises and cages, handlers should wear protective clothing, gloves and respirator mask (N-95). Examination of carcasses or any material that could be infected should be made in a biohazard cabinet (Schlossberg, 2000; Smith et al., 2003). The organism must be handled in biosafety level (BSL) 3 containment due to its zoonotic implications (Smith et al., 2005). A physician's advice is must for any person in contact with asymptomatic birds and thereby developing clinical symptoms (Ito et al., 2002).

Conclusion and future perspectives

Because of the contagious nature of avian chlamydiosis caused by *Chlamydophila psittaci* and its zoonotic implications it has gained much attention among the veterinarians as well as health authorities in recent years. New diagnostic methodologies act as driving forces to hasten identification of the disease caused by this obligate intracellular pathogen. Survey concerning the seroprevalence of avian chlamydiosis can help to avoid underestimating the prevalence of the disease in any geographical location. Along with this, molecular epidemiological studies further can strengthen the procedure of identifying the risk factors associated with people exposed to occupational hazard because of its zoonotic implication *viz.,* zoo and construction workers and even avian veterinary practitioners. Extending the treatment period is important to combat infection. Early detection of the disease together with culling of the infected birds is always recommended in order to avoid use of larger doses of antibiotics like tetracycline for therapeutic purpose. OvoTF has anti-chlamydial effect *in vivo* and aerosol administration of this natural antimicrobial protein will prove as a fruitful strategy to prevent *C. psittaci* infection especially in turkey but certainly requires further research. It is of utmost importance that development of vaccines must be focused primarily on protecting against the highly virulent strains of the organism because of their detrimental nature to the first line of defense of the host system. In terms of safety and stability particularly the DNA vaccination holds much promise against *C. psittaci* infection in avian but unfortunately effectiveness is less in human. For this reason there is still need of much of the research for improving plasmid DNA delivery as well as to express and present antigen for ensuring induction of effective immune response. The complex nature of the epidemiology and transmission pattern of the disease is the main challenge. Thus, knowing the determinants of the disease will ultimately be beneficial for implementing correct control strategies. Above all it is desirable that every possible effort need to be made to create public awareness regarding the health risk as well as regarding the economic threat possessed by the disease causing substantial loss to the farmers associated with rearing turkey and duck.

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