

AVIAN CHLAMYDIOYSIS IN TURKEYS (*Meleagris gallopavo*) IN BOSNIA AND HERZEGOVINA

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Chlamydiosis is a contagious disease of birds, mammals, reptiles and humans. So far it was demonstrated in 469 species of birds and among them, turkeys are the most susceptible domestic poultry species. The disease appears in epizootic form in intensive turkey farming. Since commercial poultry rearing is under-developed in Bosnia and Herzegovina, our investigation was based on extensively reared turkeys. Cloacal and oropharyngeal swabs were taken from 26 birds and infection was proven by common chlamydial LPS antigen detection tests (IDEIA and CW). We have used rRT-PCR technique targeting chlamydial ompA gene region in order to prove Chlamydia species. Five birds, (19.2%) were found positive as judged by IDEIA and CW tests. Among them one was positive Cp. psittaci species-specific rRT-PCR, ompA gene.

Key words: avian chlamydiosis, lipopolysaccharide (LPS) antigen, ompA gene, swab samples, turkey

INTRODUCTION

Chlamydiosis (synonyms: psittacosis, ornithosis) is a highly contagious infectious disease of birds, mammals, reptiles and humans caused by bacteria *Chlamydophila psittaci* (*Cp. psittaci*). Earlier, it was mentioned as parrot fever, parrot disease, pneumonia pseudotyphosa, *Bedsonia* and *Myagawanella* infections (Eugster, 1980).

Chlamidiosis was diagnosed around the world including the neighbouring countries (Magnino et al., 2009). Disease may affect sheep, goats, cattle, pigs, horses, cats, dogs, rabbits, mice, guinea pigs and wild mammals. It was described in amphibians, reptiles and the causative agent was isolated from ticks and other parasites. Chlamydiosis affects 460 species belonging to 30 orders of birds, and numerous studies demonstrated that turkeys are the most susceptible species among domestic poultry. Infection often occurs by inhalation causing respiratory disorders, but clinically healthy animals may shed pathogens via droppings and nasal discharge intermittently for many years. Shedding can be

activated by stress factors, including relocation, shipping, crowding, chilling and breeding (Andersen and Vanrompay, 2008; Smith et al., 2010).

In the environment *Cp. psittaci* can remain infectious for months. The usual duration between exposure to *Cp. psittaci* and onset of illness ranges from 3 days to several weeks. However the active disease can appear with no identifiable exposure. The disease in birds usually occurs in acute, rarely in chronic form, as respiratory or systemic infection. Bird species, virulence and dose of the strain, stress factors, age and the extent of treatment or prophylaxis all influence disease development and outcome (Andersen and Vanrompay, 2008). Signs of avian chlamydiosis are non-specific and include ruffled feathers, lethargy, anorexia, emaciation, dehydration and death. In some cases serous or mucopurulent ocular or nasal discharge, diarrhea and excretion of green to yellow-green urates are present. Presently, doxycycline is the drug of choice for treating birds with avian chlamydiosis (Smith et al. 2010).

Humans are usually infected by close contact with birds or by staying in places where a large concentration of birds can be encountered such as public areas, animal exhibitions, farms and competitions (Heddeema et al., 2006; Gaede et al., 2008). The infection usually occurs when a person inhales the bacterium that has been aerosolized from dried feces or respiratory tract secretions of infected birds (Telfer et al., 2005; Smith et al., 2010). The disease in humans causes typical influenza-like symptoms and can lead to severe pneumonia and non respiratory health problems. Disease can present occupational hazard for breeders of pet birds and pigeons, zoo workers, employees in shops for pet birds and in poultry facilities, veterinarians and veterinary technicians (Andersen and Vanrompay, 2008; Smith et al., 2010).

Bearing in mind the susceptibility of turkeys to *Chlamydophila psittaci*, as well as their close contact with people due to commercial importance, the research of chlamydiosis prevalence in this species of poultry has a great epizootiological importance, especially since further development of commercial turkey breeding is expected.

Chlamydiosis in turkeys caused considerable economic consequences in the United States during 1951-1956 period, especially when major epizootics occurred in 1974 in Texas and 1986 in Minnesota. Besides the United States, major enzootics have occurred in Europe: Czech Republic, Slovakia, Denmark, UK (Andrews et al. 1981), Germany and Hungary (Eugster, 1980). In Khuzestan province, 270 turkeys were tested for antibodies to *Cp. Psittaci* using ELISA test (Immunocomb-ILS). According to results of this study, the seroprevalence of chlamydiosis were 58.9% in tested turkeys (Ghorbanpoor and Myahi, 2008).

The aim of this study was to obtain a snapshot of the epidemiological situation regarding the presence of chlamydiosis in turkeys in Bosnia and Herzegovina, especially as there are no previous epizootiological data. Since commercial poultry rearing is under-developed in Bosnia and Herzegovina, extensively reared turkeys were sampled.

MATERIAL AND METHODS

A two sets of cloacal and oropharyngeal swabs from each of 26 turkeys (*Meleagris gallopavo*) were sampled between October 2007 and April 2008 in the northern and northeastern part of BiH (municipalities Orašje, Odžak, Bosanski Šamac, Živinice and Đurđevik).

Until processing, the samples were immersed into 2SP storage medium (containing 0.2 mol/L sucrose in 0.02 mol/L phosphate buffer supplemented with 10% fetal calf serum) and stored at -20°C.

Detection of specific chlamydial antigen

The first set of samples was tested for the presence of the common lipopolysaccharide (LPS) in the outer bacterial membrane of the genera *Chlamydia* and *Chlamydophila* using the IDEIA™ PCE Chlamydia Test (DAKO Diagnostics Ltd., UK) (IDEIA) according to the instructions of the manufacturer. Heating step (15 minutes in a water bath at 95°C) solubilized any chlamydial LPS present in the samples. Three formalin-inactivated chlamydial antigens in buffer solution and universal transport medium without the chlamydial antigen were used as positive and negative controls, respectively. Optical density (OD) values were measured at 490 nm using MRX microtiterplate reader (Dynatech Laboratories, GB). Evaluation of the OD values was performed following the calculation instructions provided by the manufacturer.

Additionally, a second set of samples was tested using the commercially available immunochromatographic test Clearview Chlamydia MF (Unipath Limited, Priory Park, Bedford, MK44 3UP, England) (CW), following the manufacturer instructions. This test for direct detection of *C. trachomatis* is designed for humans in the first place, but the procedure is also suitable for direct evidence of chlamydia LPS antigen in birds (Wilsmore and Davidson, 1991; Vazquez-Cisneros et al., 1994). The test uses monoclonal antibodies (Mab) directed against the genus-specific epitope located on the chlamydial LPS.

Bacteriology

Differential diagnostic testing of all samples, particularly for the *Salmonella* spp. and *Streptococcus* spp., was included to eliminate possible cross-reactions, as described elsewhere (Karakasevic et al. 1967, Erski-Biljic and Dobric 1998, Rasidbegovic and Kavazovic 2008).

DNA isolation and real-time polymerase chain reaction (rRT-PCR)

DNA isolation was performed from the samples which, according to the above described tests, were positive for chlamydia, using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) as described in the kit protocol.

Amplification of the target *ompA* gene region by rRT-PCR was carried out at the Friedrich Loeffler Institute Jena (Institute for Molecular pathogenesis of Jena, Germany). on an ABI PRISM 7000 thermocycler (Applied Biosystems), as previously described (Pantchev et al., 2009) using the 2 x TaqMan Universal PCR Master Mix supplemented with ROX (Applied Biosystems) including an internal

amplification control (IAC), specific primers and probes targeting the *ompA* gene region (Table 1), and following cycling parameters: an initial cycle of heating at 95°C for 10 min (single denaturation step), 45 cycles of 95°C for 15 s and 60°C for 1 min (annealing and extension). The cycle threshold value (*Ct*) was calculated automatically. The above-described procedure, with the same kits, primers and probes, was successfully applied on rRT-PCR platform StepOnePlus (Applied Biosystems) at Avian Diseases Department, Veterinary Faculty Sarajevo, Virology Laboratory, and identical results were obtained.

Table 1. Specific primers and probes used for detection of *Cp. Psittaci* (adapted from Pantchev et al., 2009)

Designation	Nucleotide sequence (5' – 3*)	Amplicon size (bp)
CppsOMP1-F	CAC-TAT-GTG-GGA-AGG-TGC-TTCA	76
CppsOMP1-R	CTG-CGC-GGA-TGC-TAA-TGG	
CppsOMP1-S	FAM ¹ - CGC-TAC-TTG-GTG-GAC-TAMRA ²	
Internal amplification control		
EGFP-1-F	GAC-CAC-TAC-CAG-CAG-AAC-AC	177
EGFP-10-R	CTT-GTA-CAG-CTC-GTC-CAT-G C	
EGFP-HEX	HEX ³ -AGC-ACC-CAG-TCC-GCC-CTG-AGC-A-BHQ ⁴	

¹ – FAM – 6-Carboxyfluorescein; ² – TAMRA 6-carboxy-tetramethylrhodamin;

³ – HEX – Hexachlorofluorescein; ⁴ – BHQ – Black Hole Quencher

RESULTS AND DISCUSSION

Bacteriological examination of samples

Bacteriological examination of cloacal and oropharyngeal swabs of turkeys showed no presence of *Salmonella* spp. and *Streptococcus* spp. pathogens.

IDEIA and CW tests

Of the 26 birds tested by IDEIA and CW tests for the detection of LPS antigen, five birds, or 19.2% cases, were positive.

Real-time PCR (rRT-PCR)

Samples that tested positive on IDEIA and CW tests were further tested using rRT-PCR targeting chlamydial *ompA* gene region. From a total of five tested birds, only one, or 20.0%, was positive.

Considering that chlamydiosis occurs in a large number of domestic and wild birds, which is especially important in commercial poultry production, and may be of health concern for other animal species and humans, the study was conducted on turkeys as the most susceptible commercial poultry.

Turkeys and ducks are the most important transmitters of infection to humans (Eugster, 1980; Evermann, 1987; Schachter, 1989). Before the discovery

of antibiotics, chlamydiosis was often a fatal infection in humans, with mortality over 20% (Bourke et al., 1989; Schachter, 1989). Bourke et al. (1989) and Mian et al. (1992) also described cases of *Cp. psittaci* transfer among humans.

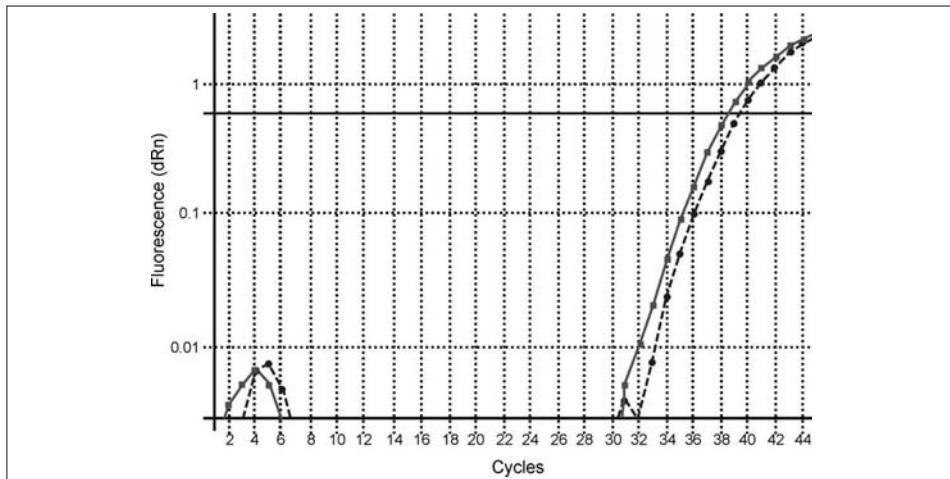


Figure 1. Amplification diagram of analysis of *ompA* gene for *Cp. psittaci* of oropharyngeal swab sample of turkey

Chlamydiosis in birds is often a systemic disease and infection can be unapparent, severe, acute or chronic with intermittent shedding. Clinically unapparent, a latent infection is supposed to be the predominant state, particularly in wild birds, which, due to this fact, have a special importance in the chain of transmission. They often have a persistent infection with no clinical signs, but with periodic emissions of pathogens into the environment which allow the infection of other animal species and humans. Contact of wild birds with farm animals is largely indirect, via contaminated food, space and equipment (Travniček et al., 2002; Šatović, 2006).

Birds are usually infected with low and high virulent strains of *Cp. Psittaci*. From the epizootiological aspect, it is important to note that both strains have the same capacity and speed of spreading within the flock. Younger birds are generally more susceptible than the older ones. Infection in older turkeys can go unnoticed, unless the birds are exposed to stressful situations. Males usually have higher mortality than females (Andersen and Vanrompay, 2008).

Serotypes of lower virulence cause small-scale epidemics with mortality rates below 5%. These strains are often isolated in pigeons, ducks, occasionally in turkeys and also in wild birds. In turkeys, lower virulence serotypes (B and E) generally do not generate clinical manifestations (Tappe et al., 1989).

High virulent strains are often isolated in turkeys (Hinz et al., 1992) in some cases, and also in other apparently healthy wild birds. Most of the isolates over the last 40 years belonged to serotype D and B of *Cp. psittaci*. B serotype was isolated

most frequently in pigeons, including in apparently healthy birds (Andersen, 1991). Reservoirs of D serotype are not known yet. Serotype D is commonly isolated in cases of rapid infection outbreak with high mortality rate. While there are indications that the infectious agent enters the flock from the outside, studies did not prove links with other bird species besides turkeys (Andersen and Vanrompay, 2008). While the vertical transmission of chlamydia is relatively rare, it still represents an important route. This type of transmission is described in turkeys flocks with birds having mild clinical symptoms. (Lublin et al., 1996; Vanrompay et al., 1995).

In our investigation IDEIA and CW tests were used for the detection of the antigen from oropharyngeal and cloacal swabs. The tests were intended for the detection of the antigen against *C. trachomatis* in human urethral and endocervical swabs and in urine and ophthalmic specimens (Vanrompay et al., 1994). Both assays contain a MAb directed against the genus-specific chlamydial LPS antigen. As such, they are suitable for the detection of *C. psittaci* infection in birds (Vanrompay, 1994; Phong et al., 1996; Schnebel, 2004).

We demonstrated the presence of chlamydial LPS antigen by both tests in 19.2% cloacal swabs. It is interesting to notice that the same percentage of the oropharyngeal swabs was positive as well. Using IDEIA test, Vanrompay et al. (1994) reported somewhat lower detection level (10%) in cloacal swabs of turkeys, compared to our results. With the same test of conjunctival and cloacal swabs of migratory birds, Schnebel (2004) detected *C. psittaci* in 38.1% samples.

Using CW test Vanrompay et al. (1994) confirmed the presence of the antigen in 20% of conjunctival and cloacal swabs from turkeys, which approximately matches our results. Rešidbegović et al. (2006) detected the presence of chlamydial LPS antigen in 16.7% pigeons from the city market and in 12.5% commercial broiler chickens, while Phong et al. (1996) found 78.3% positive pigeons and 28.6% parrots.

Previous studies showed a possibility of cross-reaction with gram-negative bacteria (Beller, 1991) and suggested careful estimation of the positive results of cloacal swabs obtained by IDEIA and CW. For that reason, we included differential diagnosis by culturing *Salmonella* spp. and *Streptococcus* spp. from cloacal swabs and these results were negative.

In order to determine species of chlamydia detected by IDEIA and CW in five positive birds, species-specific rRT-PCR targeting *ompA* gene region of *Cp. Psittaci* was used for further research. Namely, rRT-PCR has been proven as an effective diagnostic technique for rapid, specific and sensitive diagnosis of chlamydial infections (Sache et al., 2008). The sensitivity and specificity of this method are directly dependent on the procedure of sample preparation, DNA extraction and its eventual transport and used PCR test (OIE, 2004).

In our research, from a total of five turkeys tested using *Cp. psittaci* species-specific rRT-PCR, one (20.0%) was positive. This is somewhat a lower finding than 33% positive samples (commercial chicken and free living pigeons) by PCR method reported by Šatrović (2006). Using the same method in Eastern Germany, Schettler et al. (2003) found considerably higher percentage (74%) of *Chlamidia* positive wild birds.

Our results indicate the need for further systematic monitoring and research of chlamydial infections in turkeys in Bosnia and Herzegovina, especially bearing in mind their high susceptibility *Chlamydia* and their importance as vectors of infection to other commercial poultry species and humans.

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AVIJARNA HLAMIDIOZA ĆURKI (*Meleagris gallopavo*) U BOSNI I HERCEGOVINI

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SADRŽAJ

Hlamidioza je zarazna bolest ptica, sisara, gmizavaca i ljudi. Prema dosadašnjim podacima utvrđena je kod 469 vrsta ptica, među kojima su čurke najosjetljivija vrsta domaće peradi. Brojni su izvještaji o čestoj pojavi epizootija u intenzivnom uzgoju čurki. Obzirom da je komercijalno držanje čurki u Bosni i Hercegovini u povoju, ispitivani su uzorci poreklom od ekstenzivno uzgajanih ptica. Israživanje je provedeno uzimanjem kloakalnih i orofaringealnih briseva nakon čega su primjenjeni dijagnostički postupci u cilju dokazivanja infekcije testovima za dokazivanje zajedničkog hlamidijalnog LPS antiga (IDEIA i CW) te ompA gen koji je vrsno-specifični rRT-PCR za dokaz vrste hlamidije. Od ukupno 26 ptica testiranih IDEIA i CW testovima, pet ili 19,2% bilo je pozitivno. Od ukupno pet ptica testiranih *Cp. psittaci* virusno-specifičnim rRT-PCR samo jedna ptica (20%), je bila pozitivna.

