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THE PREVALENCE OF AVIAN CHLAMYDIOSIS (*Chlamydophila psittaci*) IN BOSNIA AND HEZEGOVINA

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In the article are presented the results of our research on chlamydophilosis in parrots, free-living and breeding pigeons, and intensive breeding chickens in Bosnia and Herzegovina. For detection of the antigen two immunoenzyme tests for the detection of antibodies against Chlamydophila psittaci and a complement fixation test by a Kolmer and indirect immunofluorescence method (BioMerieux, France) were used.

From a total of 275 samples of cloacal swabs the presence of Chlamydophila psittaci antigen was detected by ELISA (DAKO Ltd., United Kingdom) in 34.9% birds: 45.5% in intensive breeding chickens, 12.1% in free-living pigeons and 8.0% in parrots. By the same method the presence of Chlamydophila psittaci antigen in breeding pigeons was not detected. Sixty cloacal swabs from intensive breeding chickens and pigeons were tested by immunoenzyme test (Unipath Limited, England) and the presence of the pathogen was found in 6.7% cases. Fifty-eight sera from free-living pigeons and intensive breeding chickens were tested for the presence of specific antibodies to Chlamydophila psittaci by indirect immunofluorescence method and were found in 42.1% examined sera of pigeons, and in 27.6% pigeons from the total number of examined birds. The presence of specific antibodies was not found in sera of intensive breeding chickens. Using a complement fixation test, antibodies were not detected in the examined sera in pigeons nor in intensive breeding chickens.

The results of this study show that the presence of antigens and antibodies for Chlamydophila psittaci is obvious in tested sera samples, but the clinical disease was not found in any of the examined birds.

Key words: chlamydia infections-epidemiology, Chlamydophila psittaci, Bosnia-Herzegovina, laboratory diagnosis-methods, birds

INTRODUCTION

Avian chlamydiosis is caused by bacterium *Chlamydophila psittaci* (*C. psittaci*) (OIE, 2004; Andersen *et al*., 2003). The pathogen (or its antibodies) have been found in more than 469 different species bird (Kaleta and Taday, 2003). In birds, *C. psittaci* produces a systemic and occasionally fatal disease. Depending on the chlamydial serovar, species and age of the birds clinical signs can vary greatly in severity. The occurence of avian chlamydiosis in commercially raised poultry for eggs and meat – turkeys and ducks has been reported (Andersen *et al*., 2003). In turkeys and ducks clinical signs include lethargy, ocular and nasal discharge, egg production decrease. Mortality can be up to 30% (Woldehiwet, 2001; Andersen *et al*., 2003). In pet birds, clinical sings include sinusitis and respiratory problems, yellow-green droppings, anorexia, and loss of body weight. A number of bird species, especially elderly parrots, show no clinical signs, but can shed chlamydiae intermittently for many years. In infected birds the enlargement of the spleen and liver, fibrinous airsacculitis, peritonitis and in turkeys often pericarditis can be observed (Vanrompay *et al*., 1995; Woldehiwet, 2001; Andersen *et al*., 2003).

The strains of avian chlamydiae can infect humans. Most infections occur by handling and during the examinationes of infected birds or by inhaling contaminated airborne particules. Workers in slaughterhouses and processing plants, veterinarians, poultry farmers and particularly parrots and pigeons breeders present the greatest risk groups. The disease in humans varies from inapparent to a severe systemic disease with pneumonia. In properly treated patients the disease is rarely fatal (Andersen *et al*., 2003).

In order to prevent avian chlamydiosis it is necessary to control health conditions without contact with potentially infected birds, minimizing the contact with free-living birds as they are the possible source and carrier of infection to commercially rare poultry. Implementation of biosecurity measures is essential to minimize the spreading of the infection between birds and the transmission to humans. Special attention should be directed to minimize pathogen spreading through the air, to restrict movement of people, quarantine, hygiene, radical disinfection with iodophores, formaldehyde and quaternary ammonium products (Page, 1975; Woldehiwet, 2001). Pet birds should originate from flocks free of the pathogen. Good results can not be achieved by vaccination either in avian species or humans, considering the fact that no commercial vaccine is available for avian chlamydiosis (Woldehiwet, 2001; Andersen *et al*., 2003). However, using a plasmid DNA vaccine against MOMP antigen was shown to give a good protection against the infection with respiratory signs in turkeys (Andersen and Vanrompay, 2003).

Avian chlamydiosis is found and diagnosed in free-living birds and domestic animals worldwide (Dovč, 1998; Vlahović *et al.*, 1998; Pavlak *et al*., 2000; Andersen *et al.*, 2003; Kaleta and Taday 2003; Dovč *et al*., 2005), however in Bosnia and Herzegovina similar investigations have not been carried out yet. Considering the above mentioned and the possibility of avian chlamydiosis spreading, the objective of the present study was to investigate the prevalence

and spreading of avian chlamydiosis in wild birds and domestic poultry in Bosnia and Herzegovina.

MATERIALS AND METHODS

The prevalence of avian chlamydiosis was investigated from April 2002 to November 2003 (see Table report).

Detection of specific chlamydial antigen

Cloacal swabs of 275 birds were tested by ELISA - IDEIA^{TM} PCE Chlamydia /DAKO Ltd., Denmark House, Angel Drove, Ely, Cambridgeshire, CB7 4ET United Kingdom (IDEA). For the presence of antigen against *C. psittaci* 60 cloacal swabs were tested using immunoezyme test - Clearview Chlamydia MF/Unipath Limited, Priory Park, Bedford, MK44 3UP, England (CW).

Detection of specific chlamydial antibodies

The 58 serums were tested by indirect immunofluorescence - *Chlamydia. psittaci* spot /BioMerieux, France (IIF) (Dovč 1993) and complement-fixation test – micromethod by a Kolmer (CF). For the detection of CF antibodies in tested samples of bird sera a commercial group-specific chlamydia antigen was used (Department of Virlogy, Croatian Public Health Institute) (Vlahović, 1996; Vlahović, 2000). Blood samples were obtained by venipuncture (*V. cutanea ulnaris*).

Bacteriology

Differential diagnosis included bacteriological examination of 275 cloacal swabs for *Salmonella spp*. and *Streptococcus spp*.

Species and number of tested birds for presence of antigen and antibodies against *C. psittaci*

Species	Method for detection of antigen		Method for detection of antibodies	
	IDEIA	CW	CF	IIF
Intensive breeding chickens	198	24	20	20
Breeding pigeons	19	10	0	0
Pigeons from city market	8*	6	2	2
Pigeons from the area of a bread factory	18*	7	36	36
Pigeons from farm area	7*	5	0	0
Parrots	25	8	0	0
Total	275	60	58	58

*Total number of tested free-living pigeons (N=33)

RESULTS

The presence of antigen against *C. psittaci* was detected in 34.9% (Table 1) using ELISA test and in 6.7% (Table 2) by CW test of examined cloacal swabs.

Table 1. Results of examined cloacal swabs obtained by IDEIA

Birds species	Number of tested birds	positive		equivocal**	
		Nº	%	Nº	%
Intensive breeding chickens	198	90	45.5	0	0
Breeding pigeons	19	0	0	1	5.3
Pigeons from city market	8*	1	12.5	2	25.0
Pigeons from the area of a bread factory	18*	2	11.1	0	0
Pigeons from farm area	7*	1	14.3	1	14.3
Parrots	25	2	8.0	0	0
Total	275	96	34.9	4	1.5

* Total number of tested free-living pigeons (N=33)
**Equivocal results were re-tested and the results were negative

Table 2. Results of examined cloacal swabs by CW

Birds species	Number of tested birds	positive	
		Nº	%
Intensive breeding chickens	24	3	12.5
Breeding pigeons	10	0	0
Pigeons from city market	6	1	16.7
Pigeons from the area of a bread factory	7	0	0
Pigeons from farm area	5	0	0
Parrots	8	0	0
Total	60	4	6.7

Table 3. Results of examined sera by IIF method and CF

Bird species	Number of tested sera	IIF		CF
		titer 1:40 Nº (%)	titer 1:80 Nº (%)	positive Nº (%)
Intensive breeding chickens	20	0	0	0
Pigeons from the area of a bread factory	36	14 (38.9)*	2 (5.6)*	0
Pigeons from city market*	2	0*	0*	0
Total	58	14 (24.1)**	2 (3.5)**	0

* Percentage of all positive free-living pigeons is 42.1% (N=16)
**Percentage all positive birds (pigeons and intensive breed chickens) je 27.6% (N=58)

Of 58 examined sera samples by IIF test, specific antibodies against *C. psittaci* were detected in 16 (27.6%) samples but in the same samples, CF antibodies were not detected (Table 3).

Cloacal swabs of 275 birds were bacteriologicaly examined, *Salmonella spp.* and *Streptococccus spp*. were not isolated.

DISCUSSION

In order to protect birds and public health, continual control for avian chlamydiosis is supposed to be done and with the aim to find the sources, carriers and transmitters of *C. psittaci*. Using adequate preventive control measures it could be possible to prevent spreading of infection.

In our investigation two tests (IDEA and CW) were used for the detection of the antigen in cloacal swabs. Both tests were intended for the detection of the antigen against *C. trachomatis* in human urethral and endocervical swabs. Regarding that the monoclonal antibodies used in these tests are directed against a genus – specific epitope located on the chlamydial lipopolisaccharid, they are suitable for the detection of *C. psittaci* infection. According to literature data specified tests are used for *C. psittaci* antigen detection in turkeys (Vanrompay, 1994), pigeons, doves and parrots (Phong *et al*., 1996), migration birds (Schnebel, 2004) and koalas (Wood *et al*., 1992).

During our investigation, the presence of *C. psittaci* antigen by IDEA test was detected in 34.9% cloacal swabs from different birds: 45.5% in intensive breeding chickens, 12.1% in free-living pigeons, 8% in parrots, but breeding pigeons were negative. Using the same test, Schnebel (2004) detected *C. psittaci* in 38.1% conjunctival and cloacal swabs of migration birds. Vanrompay *et al*. (1994) confirmed the presence of antigen in five of 40 tested conjunctival and cloacal swabs of turkeys. Using the same method the antigen was found in 10 of 11 urogenital swabs from infected koalas. Test sensitivity was 91% and specificity 86% (Wood *et al*., 1992).

In our investigation by rapid immunoenzyme test (CW) the presence of *C. psittaci* antigen was found in 6.7% of the tested cloacal swabs. Positive results were found in 16.7% pigeons from the city market and in 12.5% intensive breeding chickens. In all other tested birds *C. psittaci* was not detected. Using the same test Phong *et al*. (1996) found positive results in 78.3% pigeons and 28.6% parrots. Results reported by Wood *et al*. (1992) confirmed that CW is a simple, rapid and sensitive test for the detection of urogenital infection in koalas under field conditions with the highest sensitivity (91%) compared to other tests.

Beller (1991) reported that the positive results of cloacal swabs obtained by IDEA and CW tests should be carefully estimated because of cross-reaction with gram-negative bacteria. Because of that in our investigation differential diagnosis included culturing for *Salmonella spp*. and *Streptococcus spp.* of cloacal swabs. These results were negative. Vlahović *et al*. (2004) isolated *Campylobacter jejuni*-like bacteria in 1.9% and *Salmonella spp*. in 7.4% samples of free-living birds species examined.

The presence of specific IgG antibodies against *C. psittaci* was confirmed in 42.1% pigeons of the 38 sera tested by IIF test but was not confirmed in the 20 tested sera of intensive breeding chickens. All positive sera originated from the pigeons living in the area of a bread factory. This result indicates that pigeons can be a serious problem as reservoirs and transmitters of chlamydiae. Antibody titers from pigeons living in the area of a bread factory ranged from 1:40 (38.9%) to 1:80 (5.6%). Obtained results are in accordance with the results of other authors that revealed a high percentage of seropositive free-range city pigeons.

Dovč (1993) reported 66.7% serological positive free-living pigeons and 59.2% feral pigeons using IIF test. During the 11 years period (1991-2001) by the same method were detected 5.1% and 6.2% serological positive free-living birds and parrots, respectively (Dovč *et al*., 2005).

In our investigation using CF antibodies were not detected in the sera of intensive breeding chickens nor in free-living pigeons from the city market. It is known that IIF is more sensitive than CF (Dovč 1998), which can explain our results. The birds with the intestinal form of infection do not always develop humoral immunity response or they have a very low specific antibody titer. Therefore, negative serological results obtained by CF does not exclude chlamydia infection. Most probably the tested birds have chronic infection (IgG antibodies) or the intestinal form of infection.

Durand (1982) points out that low antibody titer detected in the sera of birds can be the result of recovery from a mild form of chlamydia infection or cross-reaction with gram-negative bacteria.

Gregurić *et al*. (1989) using CF micromethod, detected antibodies against *C. psittaci* in 43.9% free-living pigeons from the city area with antibody titers from 1:4 to 1:128. Vlahović *et al*. (1998) reported that 44.1% free-living and pet birds and 47.3% breeding pigeons had antibody titers from 1:8 to 1:256, detected by CF. Pavlak *et al*. (2000), using the same method, found 49.2% serologically positive pigeons from the city area.

Regarding the high variability in the prevalence of the disease, according to the results of other authors and using different diagnostic methods, an accurate estimation of pathogen transmittion is not possible (Schnebell, 2004).

Although in Bosnia in Herzegovina avian chlamydiosis dose not seem to be a serious problem, we have proved the presence of chlamydiae more than we predicted. The estimation of the achieved results for the diagnosis of infection with *C. psittaci* depends on anamnesis, clinical signs, autopsy, histological findings, previous therapy, proper sample collection, transportation and properly chosen diagnostic methods. Regarding the incomplete knowledge of pathogenicity factors of chlamydial serovar it is also very important to prove at the same time the antigen with serological tests. Latent and chronically infected birds present a serious risk to public health and they should be excluded from breeding.

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ZASTUPLJENOST HLAMIDIOZE PTICA (*Chlamydophila psittaci*) U BOSNI I HERCEGOVINI

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SADRŽAJ

U ovom radu su prikazani rezultati istraživanja raširenosti avijarne hlamidioze kod papiga, prostoživećih i uzgojnih golubova i intenzivno držane peradi na području Bosne i Hercegovine. Za dokaz antigena korištena su dva imunoenzimska testa a za detekciju antitela protiv bakterije *Chlamydophila psittaci* test komplement-fiksacije i indirektna imunofluorescencija.

Od ukupno 275 pretraženih uzoraka kloakalnih briseva ptica, prisustvo antigena za *Chlamydophila psittaci* dokazano je postupkom ELISA (IDEIA^{TM} PCE Chlamydia) u 34,9% slučajeva: i to 45,5% kod intenzivno držane peradi, 12,1% kod prostoživećih golubova i 8,0% uzoraka kod ukrasnih ptica. Kod uzgojnih golubova istom metodom nismo utvrdili prisustvo anatigena. EIA testom (Clearview Chlamydia MF) prisustvo patogena dokazano je u uzorcima kloakalnih briseva ptica (peradi i golubova) u 6.7% slučajeva od ukupno 60 pregledanih ptica.

U uzorcima 58 krvnih seruma slobodnoživećih golubova (38) i intenzivno držane peradi (20) dokazano je metodom indirektne imunofluorescencije prisustvo specifičnih antitela protiv *Chlamydophila psittaci* u 42,1% pretraženih uzoraka seruma golubova, odnosno kod 27,6% golubova u odnosu na ukupan broj pretraženih ptica. Prisustvo specifičnih antitela nije utvrđeno u serumima kod intenzivno držane peradi. Metodom RVK nismo uspjeli dokazati antitela u ispitanim uzorcima seruma.

Iz rezultata naših istraživanja evidentno je prisustvo antigena i antitela za *Chlamydophila psittaci* u ispitanim uzorcima, ali niti u jedne ptice nije bilo zabeleženo klinički manifestno obolenje.