Research of Chlamydiosis presence in dogs population in Bosnia and Herzegovina

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Abstract

Our research describe epidemiological presence of Chlamydiosis in different categories of dogs in Bosnia and Herzegovina. Problem of stray dogs, inordinately examined and not vaccinated dogs is one of the most complex problems among citizens, nongovernment organisations and institutions in Bosnia and Herzegovina. Chlamydiosis is zoonotic disease caused by Gram-negative, intracellulare bacteria, which include strains: *Chlamydophila felis*, *Chlamydophila abortus*, *Chlamydophila psittaci* and *Chlamydophila caviae*. Disease have endemic characteristics and there is little information about natural infections in dogs, which were mostly related to conjuctivitis, encephalitis and symptoms characteristic for pneumonia. In Europe, research of clamidiosis in dogs has been conducted in a small number of countries which include Germany, Slovakia, Sweden and Lithuania. This was a first of its kind study of Clamidiosis in dog population, carried out in Bosnia and Herzegovina. The study was conducted in twelve cities in Bosnia and Herzegovina with cooperation between two departments for contagious disease in Veterinary faculty of Sarajevo and Veterinary faculty of Ljubljana. The aim of the research was to determine presence of Chlamydial infections in different categories of dogs, using modern serological and molecular diagnostic methods. Blood serum samples were taken during 2012/2013. In total, 294 samples were assessed for presence of specific Chlamydial antibodies using method of indirect immunofluorescence, while method of RT-PCR was used for determination of antigen. After assessing 294 blood serum samples, 2.04% (6 samples) were positive for *Cp. psittaci*. Most of the positive samples originated from stray dogs. From serology positive animals, nose swabs were taken and assessed using RT-PCR. The presence of nucleic acid from *Cp. psittaci* was not confirmed in any of them.

Key words: *chlamydiosis; antigen; antibody; dogs; Sarajevo*

Introduction

Pets and working dogs (Shepherds, Hounds and Watchdogs) had always their traditional place among citizens in Bosnia and Herzegovina. Unfortunately, because of unfavorable economic conditions in country during last several years, there

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was a notable increase in number of abandoned dogs, regardless of their breed. However, many citizens still kept their dogs, but could not perform regular health inspections or vaccinations, where together with abandoned dogs as a result, unfavorable epizootiological situation has developed. Anadolu Agency quotes that a problem of stray dogs is one of the most complex problems among citizens, nongovernment organisations (NGOs) and institutions in Bosnia and Herzegovina (BiH). There is no official information about number of stray dogs in the cities, but estimates suggests it to be around 7000 of them just in Sarajevo. (http://www.oslobodjenje.ba/vijesti/bih/psi-su-strah-i-trepet-sarajeva-lutalice-do-sada-ugrizle vise-od-dvije-hiljade-ljudi). Stray dogs has become a serious threat for public health, because their number incerase all the time and during last several years and fear among citizens is justified by the notion that a large proportion of the stray dogs are potential carriers of contagious diseases (CD), which may jeopardise health in humans and animals.

Chlamydiosis in dogs is disease caused by gram-negative, intracellulare bacteria, which include strains: *Chlamydophilia felis*, *Chlamydiophila abortus*, *Chlamydophila psittaci* and *Chlamydophila caviae* (Pantchev et al., 2010). Chlamydias are group of bacterias resistent to environmental factors such as cooling and dehydration (Gylstroff and Grimm, 1987).

Figure 1. Sampling locations in territory of Bosnia and Herzegovina.

Chlamydias with their biological and morphological characteristics looks like bacterias and viruses. However the only characteristic that chlamydias share with viruses is that they are forced obligate intracellular "parasites" and use the hosts ATP, as well as guanosine triphosphate (GTP) (Acha and Szyfres, 2003). Unlike viruses, chlamydias have cellular membrane, similar to gram-negative bacterias, contain DNA and RNA, have own ribosomes, synthetize their own proteins, nuclein acids and fats and are responsive to tetracyclins as well as certain antibiotics that inhibit their multiplication (Moulder, 1984).

Chlamydias were found and described, for the first time in dogs more then 50 years ago in Germany (Sprague et al., 2009). Disease have endemic characteristics and there is little information about natural infections in dogs, which were mostly related to conjuctivitis, encephalitis and symptoms characteristic for pneumonia (Gresham et al., 1996).

Clinical cases of chlamydiosis are manifested via increased body temperature, apathy, vomiting, difficult breathing, uncoordinated movements, conjuctivitis, enteritis, joint pains and neurological disorders (Greene and Sykes, 2006; Liutkeviciene et al., 2009). Researches has found that pathogenicity is dependant of toxin potency (Chahota et al., 2006; Corsaro and Greub, 2006).

Importance of chlamydiosis is also zoonotical. Several types of *Chlamydiales* can be transmitted from animals to humans. Most often it is: *Cp. psittaci, Cp. abortus* and *Cp. felis*. Humans infected with *Cp. psittaci* may result in symptoms similar like atypical pneumonia, sometimes with complications like endocarditis,

miocarditis, hepatitis, encefalitis, artritis, keratoconjunctivitis (Chau et al., 2015), while infection with *Cp. abortus* in women may cause miscarriage (Smith et al., 2005). The greatest zoonotic potential in dogs develop *Cp. psittaci* (Public Health England, 2014), but zoonotic potential also have *Cp. felis*.

Transmission is most likely through air, as well as by eye or nose secretions from diseased cats and dogs. Clinical cases of Chlamydiosis in humans caused by *Cp. felis* is mostly manifested with conjunctivitis. Sometimes, there are cases with complicated additional clinical symptoms (Browning, 2004; Wu et al., 2013).

Because of potential for spreading disease from animal to humans (zoonosis), presence of Chlamydiosis both in animal and public, increased number of stray dogs, reduced number of vaccinated pets and working dogs and insufficient data about widespread of this disease, were the main reasons for the research study about the presence of Chlamydiosis in the stray dog population in Sarajevo. The need for further research comes from the fact that Bosnia and Herzegovina is a country in transition with the intention of joining the European Union (EU). Implementation of measures for monitoring of contagious diseases, especially zoonosis, their prevention and therapy is one of the preconditions for joining EU.

Materials and methods

The study into the presence of Chlamydiosis in dogs population was conducted in period from September 2012 to August 2013 and it involved in total 294 dogs of different breeds, 197 male and 97 female, age 6 months to 10 years. Our study included twelve cities: Busovača, Goražde, Jablanica, Ključ, Konjic, Lukavac, Prijedor, Sanski Most, Sarajevo, Travnik, Tuzla and Zenica. Dogs were divided in three categories: 1. stray dogs; 2. pets; 3. working dogs. The overall study was conducted between two departments for contagious disease in Veterinary faculty of Sarajevo and Veterinary faculty of Ljubljana. Method applied for verifying specific clamidial antibodies was Indirect Immunofluorescence (IIF), while Real Time-Polimerase Chain Reaction (RT-PCR) was used for proving antigens. In addition, in the current study, clamidial antigen were verified using nose swab samples in cases of serology positive dogs.

Blood samples were taken in vacutainer tubes, containing etilen diamin tetraacetate (EDTA) (BD Vacutaner Systems, Belliver Industrial Estate, Plymouth, UK). Blood samples were transported in temperature of +4 °C to laboratory. In the laboratory, blood was immidiately centrifugated (4000 rpm for 15 minutes, LC 320) to separate the serum. Separated serum was transferred with pipette to sterile eppendorf tubes and stored in a freezer (-20 °C), where it was held until transportation to Veterinary faculty of Ljubljana. Before samples of nasal epithelial cells from dogs were taken, nasal surroundings were cleaned with sterile gauze to remove dirt. Sampling swab was then dipped in 2-sacharose-phosphate medium for purposes of transport (2SP) that lasted 10 to 20 seconds. 2SP contain sacharose (74.6 g/L), KH_2PO_4 (0.512 g/L), K_2HPO_4 (1.237 g/L) and L-glutamin acid (0.721 g/L). Following this, a swab was used (Clearview Chlamydia, Dacton-Tipped swabs, UK) to "wipe" dog's nasal mucous membrane. During wiping, special care was taken not to touch skin or hairs arround nostrils, which could potentially contaminate the sample. Every swab with nasal epithelial cells was dipped in eppendorf tube, filled with transport medium for clamidia. These samples were again transported to

laboratory of Department for Contagious Diseases with Epizootiology department for in Veterinary faculty of Sarajevo, where they were frozen (-20 °C), until transportation and PCR processing in laboratory of Institute for Poultry, Birds, Small Mammals and Reptiles in Veterinary faculty of Ljubljana, Slovenia.

The sampling methology of the biological material, was performed in accordance with the law for animal welfare and protection (Official Gazette of the Bosnia and Herzegovina no. 316/09).

Results

Most of the animals had relatively good general health status, but in 17.3% certain pathological conditions were observed, like eye and nose discharge, urinary, digestive, respiratory and reproductive problems and other minor health disorders.

Presence of specific antibodies IgG for *Cp. psittaci* were conducted using method of IIF. Out of 294 tested serums, 2.04% (6/294) were positive. None of the six serologicaly positive animals, had any clinical manifested signs of disease.

Titar of proven antibodies for *Cp. psittaci* had range from 1:80 to 1:640. In three dogs, determined titar was 1:80, in two dogs was 1:160, while in one dog determined titar was 1:640.

In the seropositive dogs, chlamydial infection could not been confirmed using method of nasal swabing and molecular detection.

RT-PCR was used for direct measurement of chlamydia, from nasal

Table 1. Representation of seropositive and seronegative dogs according to categories.

Categories	Stray dogs (*n*=107)	Pets (*n*=98)	Working dogs (*n*=89)
Positive dogs	3.73% (*n*=4)	1.02% (*n*=1)	1.12% (*n*=1)
Negative dogs	96.27% (*n*=103)	98.98% (*n*=97)	98.88% (*n*=88)

Table 2. Number and percentage of seropositive animals to *Chlamydophila psittaci* according to dilutions and cities

	Titar IgG antibodies of *Chlamydophila psittaci*					
CITY	1:40	1:80	1:160	1:320	1:640	% positive
Busovača (*n*=9)	0	0	0	0	**1**	**11.1**
Goražde (*n*=17)	0	0	0	0	0	0
Jablanica (*n*=8)	0	0	**1**	0	0	**12.5**
Ključ (*n*=11)	0	0	0	0	0	0
Konjic (*n*=20)	0	0	0	0	0	0
Lukavac (*n*=32)	0	0	0	0	0	0
Prijedor (*n*=11)	0	0	0	0	0	0
Sanski Most (*n*=23)	0	0	0	0	0	0
Sarajevo (*n*=59)	0	**3**	**1**	0	0	**6.8**
Travnik (*n*=48)	0	0	0	0	0	0
Tuzla (*n*=31)	0	0	0	0	0	0
Zenica (*n*=25)	0	0	0	0	0	0
TOTAL (*n*=294)	0	3	2	0	1	2.04

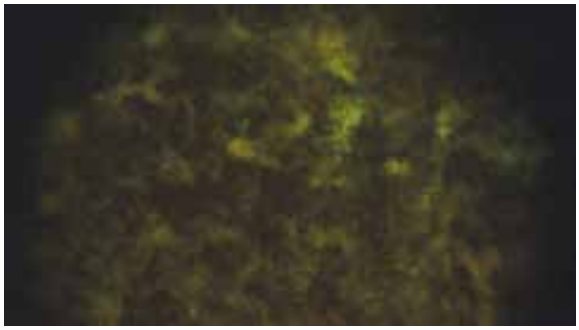

Figure 2. Photo of negative result after assessment of blood serums samples, using method of IIF and tied antigen of *Cp. psittaci* on glass slide (microscop enlargement 400 x).

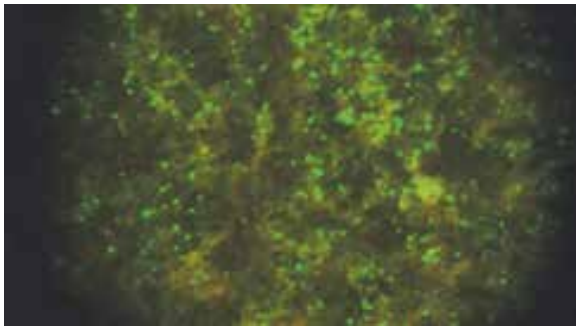

Figure 3. Photo of positive result after assessment of blood serums samples, using method of IIF and tied antigen of *Cp. psittaci* on glass slide (microscop enlargement 400 x).

swabs, taken from animals, that were serologicaly positive. In none of the six serologicaly positive animals, nucleic acid of *Cp. psittaci* could be confirmed, using this method.

Figures 2. and 3. shows negative and positive serology results with IIF method, using tied *Cp. psittaci,* antigen on slide and magnification 400 x.

Discussion

All animals as well as humans are susceptible to chlamidial infections. Chlamydiosis in dogs has been insufficiently investigated in terms of its: pathogenesis, clinical condition, diagnostics, as well as contamination rates and prevalence between animal species.

In Europe, research of chlamydiosis in dogs has been conducted in a small number of countries which include Germany, Slovakia, Sweden and Lithuania (Kocianova et al., 1992; Liutkeviciene et al., 2001; Liutkeviciene et al., 2009; Holst et al., 2010; Takačova et al., 2010). Their prevalence rates were in ranges from 0% in Sweden to 38.1% in Lithuania Research into the epizootical appearance of chlamydiosis have been conducted in Asia and North America with seroprevalence range from 9.4% to 12.1% (Fukushi et al., 1985; Arizmendi et al., 1992; Wu et al., 2013).

Chlamydiosis in dogs could be described as one of the best „impersinator" among infective diseases, given that it can look very similar to other contagious diseases, which with their clinical condition „mask" noncharacteristic clinical signs of chlamydiosis (Liutkeviciene et al., 2009). In the same manner, chlamydiosis is followed by polymorphism of clinical signs, which can result in confusion in terms of diagnostics and therapy. Because of mentioned facts, chlamydiosis in dogs is differentially difficult to separate from rhinitis and keratoconjunctivitis. Another reason for the need of fast and accurate diagnosis of chlamydiosis in dogs is the lack of standardized diagnostic procedures, diagnostics for routine testing, as well as source of adequate information.

Indirect immunofluorescence (IIF) is one of the serological methods for diagnosing Chlamydiosis in dogs. This method is based on marking specific antibodies with fluorescent colour – fluorohromom, which is covalently tied with antibodies specific for chlamydia. As such this technique allows for recording of the disease, without changes in its functionality and immune specificity (Owen et al., 2003). Molecular methods of chlamydial diagnostics are based on use of PCR, the most common used method for amplification of nucleic acids. Such

procedure is sensitive and specific, while based on segmental DNA replication. PCR procedure allows exact verification of nucleic acids from causative agent in biological materials, taken from animals. In addition to classic PCR, real-time PCR (RT-PCR) is also often applied.

Considering there is an absence of mandatory control for chlamydiosis in dogs, the presence of this disease had not been researched previously in Bosnia and Herzegovina and this is the first of its kind study performed with the main aim to determine the presence of chlamdial infections in the population of stray dogs in larger cities. The serology assessment (IIF) of blood serum in dogs (n=294), found seroprevalence of chlamydiosis to be 2.04% (6/294). Serology positive dogs however did not have clinically manifested signs of the disease, neither did they have signs of other contagious disease or disorders. By using molecular diagnostics, we could not prove the presence of chlamydia in serologically positive animals. Given the negative results in terms of the presence of antigen, as well as the absence of clinical signs of the disease, we can assum that these dogs do not secrete antigen in their surroundings, as they are not their immediate source of infection. Presence of antibodies, without confirmed presence of antigen, leads us to assume that dogs who had contact with antigen, have developed immunological reaction, but they do not inevitably have to become ill or be a threat to other animals and public health.

Research of chlamydial infections in dogs, conducted in other countries so far (Kocianova et al., 1992; Liutkeviciene et al., 2001; Liutkeviciene et al., 2009; Holst et al., 2010; Takačova et al., 2010) have covered bigger regions and larger samples, it is therefore difficult to draw a parallel between our and other research across the world. However, it does leaves us enough space to continue research of this infection in wider territory of Bosnia and Herzegovina. As prophylactic measures, increased zootechnic procedures could be used for the purpose of breeding and lodging (birds, cats, dogs), as well as routine monitoring of their health condition and development of vaccine to build up immunity in animals.

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Istraživanje klamidioze u populaciji pasa lutalica u Bosni i Hercegovini

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Naše istraživanje opisuje epidemiološko prisuće klamidioze u različitim kategorijama pasa u Bosni i Hercegovini. Problem pasa lutalica koji nisu redovito pregledavani i necijepljenih jedan je od najkompleksnijih problema među građanima, problema nevladinih organizacija i institucija u Bosni i Hercegovini. Klamidioza je zoonotska bolest koju izaziva Gram-negativna, intracelularna bakterija, a koja uključuje sojeve: *Chlamydophilia felis*, *Chlamydophilia abortus*, *Chlamydophilia psittaci* i *Chlamydophilia caviae*. Bolest je endemičnog karaktera i malo je dostupnih informacija o prirodnim infekcijama u pasa, koje su obično bile povezivane s konjuktivitisom, encefalitisom i simptomima karakterističnim za pneumoniju. Mali je broj europskih zemalja istraživao klamidiozu u pasa. Istraživali su ju: Njemačka, Slovačka, Švedska i Litva. Ovo je prvo istraživanje ovog tipa, provedeno na psima u Bosni i Hercegovini. Istraživanje je provedeno u dvanaest gradova Bosne i Hercegovine u suradnji odjela za zarazne bolesti Veterinarskog fakulteta u Sarajevu i Veterinarskog fakulteta u Ljubljani, Slovenija. Cilj je istraživanja bio uporabom metoda serologije i molekularne dijagnostike odrediti prisuće klamidijalnih infekcija među različitim kategorija pasa. Krvni serumi su uzorkovani tijekom 2012./2013. godine. Ukupno je pregledavano 294 uzoraka na prisuće specifičnih klamidijalnih protutijela metodom indirektne fluoroscence, dok je RT-PCR metoda korištena za određivanje antigena. Nakon pregleda 294 uzoraka seruma, 2,04 % (6 uzorka) su bila pozitivna na *Cp. psittaci*. Većina pozitivnih uzoraka je imala podrijetlo od uličnih pasa. Od serološki pozitivnih životinja, uzimani su brisevi nosne sluznice i pregledani uporabom RT-PCR metode. Prisuće nukleinske kiseline od *Cp. psittaci* nije potvrđena niti u jednoj od životinja.

Ključne riječi: *klamidioza, antigen, protutijelo, psi, Sarajevo*