

SEROPREVALENCE OF CAT LEPTOSPIROSIS IN BELGRADE (SERBIA)

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With its epizootiological characteristics, the territory of the municipality of Belgrade city is a potentially important locality for the maintenance and spreading of a number of serovars of *Leptospira interrogans*. In order to evaluate the epizootiological situation as far as animal leptospirosis in the Belgrade region is concerned, from January 2012 until June 2013 the prevalence of cat leptospirosis has been evaluated. The standard microagglutination test (MAT) was used to determine animals sero positive to different serovars that belong to *L. interrogans sensu lato* complex. The antigens used were: Icterohaemorrhagiae, Grippityphosa, Pomona, Canicola, Bratislava, Bataviae, Sejroe, Pyrogenes, Australis and Autumnalis. Out of the total number of tested animals, there were 43 (26.7%) positive to one, two or three serovar(s). Out of a total of 43 positive sera 20 (46.5%) samples were positive to more than one leptospira serovar.

Key words: cat, leptospirosis, microagglutination, seroprevalence

INTRODUCTION

One of the most globally important zoonotic bacterial diseases is leptospirosis. It affects both humans and animals and it is of great importance in some noso regions in the world. Leptospirosis is an acute, febrile infectious disease. In humans, the infection can range in severity from clinically unapparent to a life threatening condition. In animals, the symptoms are septicemia, icterus, as well as hepatic syndrome. In some cases, the uremic form presents itself with hemorrhagic enteritis. For dogs, cats, and partially for humans, eight leptospira serovars are of clinical and epizootiological / epidemiological importance [1,2].

Animals are reservoirs for different serovars of leptospira in particular regions. From the animal-reservoir, transmission occurs by direct or indirect contact. Direct contact is by contaminated urine, genital and/or placental secretions, via skin wounds or by ingestion of the contaminated tissues. Indirect contact, means transmission by contact

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with water, objects or any other contaminated material in the animal's environment [1]. Once infected, animals can be leptospira reservoirs for a long period. Such animals are a potential source of infection for other animals and for humans.

In spite the fact that in the local cat population there are leptospira seropositive individuals, clinical cases are seldom seen in this animal species [1]. It is generally accepted that cats are less susceptible compared to dogs not only to natural infection but to experimental infection, as well [3]. So far, successful leptospira Canicola, Grippotyphosa and Pomona serovars isolation has been achieved [4]. It has been shown that experimentally infected cats can secrete leptospira for a period of three months via urine [5].

Often, cats are exposed to contaminated dog urine. There is also the possibility for the transmission of the disease to cats from rodents that are reservoirs for serovars Balum and Icterohaemorrhagiae. Cats can be infected not only as a result of ingestion of contaminated material, but via skin wounds, also.

As far as leptospirosis in other animal species in the Belgrade region is concerned, our previous preliminary studies show that anti-leptospira antibody positive animals are horses, as well as dogs. Seroprevalence in horses was 6.29% and out of this number, positive sera to sv. Grippotyphosa, sv. Icterohaemorrhagiae, sv. Pomona, sv. Canicola, sv. Batavie and sv. Australis were detected in 2.47%, 1.12%, 0.99%, 0.94%, 0.65%, and 0.19% animals, respectively [6]. Titres of anti leptospira antibodies were in the range from 1:100 up to 1:1000. In the same epizootiological region, the total number of seropositive dogs was 14%. Seropositivity was registered to sv. Icterohaemorrhagiae (8.8%), sv. Grippotyphosa (6.1%), sv. Pomona (4.2%) and sv. Canicola (0.46%). Titers were in the range from 1:100 up to 1:400 [7].

In order to diagnose leptospira seroconversion the Microagglutination Assay Test (MAT), as well as the Enzyme Linked Immunosorbent Assay (ELISA) are in use. Moreover, MAT is prescribed as a standard for serology diagnosis of leptospirosis [8].

MATERIAL AND METHODS

Blood samples were collected from 161 cats at the Faculty of Veterinary Medicine, University of Belgrade from January 2012. to June 2013. Sampled animals were part of a project of cat hysterectomy or castration. Albeit stray cats, all animals were clinically healthy. No animal showed any symptom of leptospirosis. Blood (2.0 ml, no added anticoagulant) was collected aseptically from the *v. jugularis*. Blood samples were centrifuged (2000 g, 15min), and the obtained sera aliquoted and kept at -20°C, until testing.

Micro agglutination test was performed at the Department of Infectious Diseases of Animals and Bees, Faculty of Veterinary Medicine, Belgrade. The method followed the standard procedure [8] and was carried out in two phases. The first phase was a screening test, and the second was the final confirmation test. Ten serovars of *L.*

interrogans were used as antigens in the test: Icterohaemorrhagiae, Grippytyphosa, Pomona, Canicola, Bratislava, Batavie, Sejroe, Pyrogenes, Australis and Autumnalis. Leptospira serovars were grown in Johanson's liquid media (28°C) for 4-8 days and used as antigen (2×10^8 bacterial cells/ml, bacteria counting chamber, dark field microscope). For the screening test the same volume of diluted sera (1:50) and antigen (bacteria) suspension were added into the microtiter plate wells in order to obtain 1:100 final dilution of the tested sera. Two control sera (positive and negative) and one antigen control were used for each assay. The plates were incubated in a humid chamber (28°C, 120 min). One drop of each sample, as well as the control, was examined using a dark field microscope (100x, Carl Zeiss, Axiostar). The endpoint titer was determined as the greatest serum dilution showing agglutination of at least 50% of the leptospira. Titers of $\geq 1:100$ were considered as positive [8].

Each sample that was positive in the screening test (first phase) in 1:100 dilution, was tested in the second phase to reach the endpoint dilution of antibody positive sera (dilutions 1:200, 1:400, 1:800 and 1:1600) using the same procedure.

RESULTS AND DISCUSSION

Results of the study of seroprevalence of leptospirosis among cats in Belgrade for the first time give more details about the importance of cats for different serovars of leptospira transmission and maintenance in the urban and suburban Belgrade region. One of the most important prerequisite for leptospirosis control and eradication in the region is the knowledge on disease distribution and presence of different leptospira serovars. Although leptospirosis symptoms are seldom seen in cats, this species shares the same ecological niche with important leptospira reservoirs.

Out of 161 cat serum samples, 43 (26.7%) were found positive to at least one serovar of *L. interrogans*. As far as one serovar positive serum is concerned (Table 1), the highest prevalence was to sv. Australis (9 samples) followed by sv. Pomona (8 samples), sv. Canicola (2 positive samples), sv. Pyrogenes (2 samples), sv. Bratislava (1 sample) and sv. Grippytyphosa (1 sample). That means that seroprevalence to sv. Australis, sv. Pomona, sv. Canicola, sv. Pyrogenes, sv. Bratislava and sv. Grippytyphosa was 39.0%, 35%, 2.9%, 2.9%, 1.4% and 1.4%, respectively.

Table 1. Samples tested antibody positive to one leptospira serovar

Serovars	Serum titer	Total positive
Australis	1/100	8
	1/800	1
Pomona	1/100	8
Canicola	1/100	2
Pyrogenes	1/100	2
Bratislava	1/100	1
Grippytyphosa	1/100	1

There were 18 cat sera positive to two leptospira serovars (Table 2), and two serum samples were positive to three leptospira serovars (Table 3).

Table 2. Samples tested antibody positive to two leptospira serovars

Serovars	Number of samples and antibody titers			Total
	1	2	3	
Pomona/icterohaemorrhagiae	400/100	200/100	-	2
Bratislava/batavie	100/100	100/100	800/100	3
Pomona/canicola	100/100	100/100	100/100	3
Pomona/australis	100/100	-	-	1
Australis/sejroe	100/100	-	-	1
Australis/pyrogenes	100/100	-	-	1
Australis/Bratislava	100/100	400/100	100/100	3
Pomona/Bratislava	100/100	400/200	-	2
Bratislava/canicola	100/100	200/100	-	2

Table 3. Samples tested antibody positive to three leptospira serovars

Serovars	Titer	Total
Pomona/icterohaemorrhagiae/grippotyphosa	100/100/100	1
Pomona/australis/bratislava	100/100/100	1

Table 4. Total number, as well as proportion of seropositive cats to one, two or three leptospira serovars

Serovar	One (%)*	Two		Three	
		Serovar	Total (%)	Serovar	Total (%)
Australis	9 (39 %)	Sejroe (1)	5 (28 %)	---	---
		Pyro. (1)			
		Bratisl. (3)			
Pomona	8 (35 %)	Ictero. (2)	8 (44 %)	Ictero/gripp	1 (50 %)
		Canicola (3)		Austr./bratisl	1 (50 %)
		Austral. (1)			
Canicola	2 (9 %)	---	---	---	---
		Pyrogenes	2 (9 %)	---	---
Bratislava	1 (4 %)	Batavie (3)	5 (28 %)	---	---
		Canicola (2)			
<i>Grippotyphosa</i>	1 (4 %)	---	---	---	---
Total (%)**	23 (14.3%)		18 (11.2%)		2 (1.2%)

(*) Number (prevalence) of antibody positive sera to one, two or three serovars in relation to the total number of antibody positive sera; (**) Number (prevalence) of antibody positive sera to one, two and three serovars in relation to total number of tested sera (161).

Specific anti leptospira antibody titre (MAT) $\geq 1:100$ is considered as a sign of active or past infection or recent vaccination [9]. It is generally assumed that cats are relatively resistant to infection so this species is rarely vaccinated against leptospirosis on a routine basis. Hence, a positive MAT result is the sign of exposure to one or more leptospira serovars. In general, contact with rodents, wild animals and stray dogs can be taken as a source of infection [1].

According to data available from other regions, seroprevalence of cat leptospirosis has been investigated in Spain: 14.0% [10], [11], France: 48.0% [12], Greece: 33.3% [13], India: 66.6% [14], Scotland: 9.2% [15], Trinidad (West India): 12.5% [16], Australia: 16.9% [17], Iran - Tehran: 27.0% [18], and Ahvaz region: 4.9% [19], Southern Taiwan: 9.3% [20], Tyrol (Austria): 18.2% [21], and Brazil: Goiania region: 6.96% [22], and Bahia region: 33.3% [23].

In Tehran, out of all 111 tested cats, 19 stray and 11 in house kept cats were found antibody positive (MAT) to one or more than one *L. interrogans* serovars. The same study showed that among seropositive stray cats 97.4% were positive to sv. Canicola and 5.3% to sv. Pomona [18]. Different results were obtained in Ahvaz region of Iran where leptospira seroprevalence was low (4.9%). The highest seroprevalence was to sv. Balum (5 samples) whereas antibodies specific to more than one serovar (Balum and Australis) have been detected only in one cat [19].

Results show that leptospirosis prevalence can differ not only according to country, but to particular region also. Such differences could be the result of environmental factors and can influence serovar distribution of leptospirosis in animals and human population. That means that in different regions, cat population could be more often in contact with different animal species as a maintenance leptospira host.

The obtained results show that in the area of Belgrade city, leptospirosis is one of the highly prevalent infectious diseases of cats who potentially could serve as a significant factor in disease transmission, as well as a bacteria reservoir. The highest seroprevalence among seropositive animals has been found to sv. Australis (39.0%) and sv. Pomona (35.0%). Other serovars are of less importance especially sv. Canicola which is the major *L. interrogans* serovar, as far as dog leptospirosis is concerned.

It has been already reported [4] that sv. Canicola, Grippotyphosa and Pomona are the most common leptospira serovars isolated from cats. Results of our study show that in the Belgrade region, among the total number of seropositive animals, the highest number of antibody positive were to sv. Australis (9 animals) and sv. Pomona (8 animals). Seroprevalence to sv. Australis, sv. Pomona, sv. Canicola, sv. Pyrogenes, sv. Bratislava and sv. Grippotyphosa was 20.9%, 18.6%, 9%, 9%, 4% and 1%, respectively. These results support the basic leptospira epizootiology: serovars of *Leptospira interrogans* have different affinities for animal hosts of which distinct categories are recognized. In some regions, there are maintenance hosts that serve as a source of infection for accidental hosts.

Rarely seen, clinical symptoms of leptospirosis in cats can be the result of the adaptation of cats to particular bacteria serovars. Blood samples were collected from

cats with no clinical symptoms; moreover, there were no symptoms in cats with a high level of anti leptospira antibodies in the serum. This result supports the observation that even in the situation where cats were experimentally challenged with virulent leptospira serovars, clinical symptoms are seldom seen in spite of histological changes in target tissues such as the kidneys and liver [24].

Out of the total number of positive cat sera, 18 sera were (11.2%) positive to two leptospira serovars and two sera (1.2%) were positive to three leptospira serovars. If one wants to apply such criteria to the results in this study, the conclusion could be that the highest seroprevalence was to sv. Pomona (8 monospecific, 8 bivalent and 2 tri valent). The next highest prevalence could be to sv. Australis: 14 (9 monospecific and 5 specific to some other serovar).

It is documented that the specificity of MAT is good and there is no cross reactivity to other bacteria. However, there is significant cross serological reactivity between different leptospira serovars and serogroups. This means that an animal positive to one serovar as a result of infection, can possess cross reactive antibodies to other serovars, in lower concentrations. That is the reason why serological results cannot be the basis for leptospira serovar identification in a particular region [8].

It has been published [25] that it is possible for one animal (cow or sheep) to have a MAT positive reaction to two and/or three Leptospira serotypes. The importance of cross reactivity can be discussed. Same animal species, as in our study is the cat, can be infected in the same region at different time intervals with different leptospira serovars that are present in that region. However, it is important to stress the possibility for one serovar, being under specific herd (i.e. one species) immunity pressure, to change its antigenic structure. It has been documented that leptospirosis in cats is most often a chronic and/or inapparent infection. It is possible to obtain cross reactive cat sera if antigens obtained from a referent laboratory are used in MAT. The basis for such speculations are the epizootical characteristics of *L. interrogans* [26]. Changes of the carbohydrate moieties (glycoprotein component) in the antigenic structure of bacteria, significantly contribute to the adaptation of leptospira to different animal species in a particular region [27]. Whatever approach to the results of this study is more realistic, for further evaluation, there is a need to isolate the leptospira from seropositive cats, as well as to perform molecular mapping of the antigenic structure of the isolated bacteria.

The higher prevalence of seropositive results to different serovars of *L. interrogans* in cats (26.7%) in comparison to dogs (14.0%) and horses (6.29%) in the Belgrade region could be an indicator of more frequent effective contacts of cats with primary reservoirs in the region. Based on the results of this study, in order to obtain a complete insight into the significance of the cat population in the leptospirosis epidemiology in Belgrade city area, it is essential to perform more detailed studies, as well as to involve other animal species, rodents in particular.

CONCLUSIONS

In spite the limited number of blood samples, it has been shown that cat leptospirosis is significant in the Belgrade area. That means that this species is quite often in contact with one or more than one leptospira serovar reservoirs. Before sampling, there were no clinical symptoms observed in the sampled animals and after ovariohysterectomy, cats remain in good health. *L. interrogans* isolation was not performed so any conclusions on the epizootiological and epidemiological importance of cats as leptospira reservoirs, as well as vectors for the other animal species and human leptospirosis cannot be made. As a preventive measure, vaccination of cats as well as control of cat-rodent contacts should be considered. At the same time, cats should be kept away of locations that support the survival of different leptospira serovars (ponds, swamps, etc) [28],

These are only preliminary results as far as infection of cats with different serovars of leptospira in the wide Belgrade region, is concerned. Seroprevalence was investigated for a short period of time (16 months) and on a limited number of animals (161). In order to have a more clear picture of the epizootiological and/or epidemiological importance of cats for leptospirosis in the Belgrade region, more samples from the cat population clustered according to sex, age as well as living habitat has to be tested. Leptospira isolation from blood, urine and tissues, and molecular antigenic structure analysis is a prerequisite to have a complete information. Nevertheless, a relatively high percentage of seropositive cats found in our study, indicate that this animal species could play a significant role in the epizootiology, as well as epidemiology of leptospirosis, in the Belgrade region.

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Disclosure Statement

No competing financial interests exist.

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SEROPREVALENCIJA LEPTOSPIROZE MAČAKA NA TERITORIJI BEOGRADA (SRBIJA)

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Svojim epizootiološkim karakteristikama, teritorija grada Beograda je potencijalno značajna lokacija za održavanje i širenje brojnih serovarijeteta vrste *Leptospira interrogans*. U cilju evaluacije epizootiološke situacije u odnosu na leptospirozu kod vrsta životinja na teritoriji grada Beograda, obavljena su ispitivanja seroprevalencije leptospiroze mačaka u periodu od januara 2012. godine, do juna 2013. godine. Radi utvrđivanja prisustva antitela na različite serovarijetete leptospira, kao dijagnostički metod, upotrebljen je standardni mikroaglutinacioni test (MAT). U okviru navedene dijagnostičke metode, upotrebljeni su različiti serovarijeteti bakterije *L. interrogans* sensu lato kompleksa i to: sv. *Icterohaemorrhagiae*, sv. *Grippotyphosa*, sv. *Pomona*, sv. *Canicola*, sv. *Bratislava*, sv. *Bataviae*, sv. *Sejroe*, sv. *Pyrogenes*, sv. *Australis* i sv. *Autumnalis*. Od ukupnog broja ispitivanih životinja, pozitivnih je bilo 43 (26.7%) i to jedan, dva i/ili tri serovarijeteta. Od ukupnog broja seropozitivnih životinja, 20 seruma (46.5%) je bilo seropozitivno na više od jedan serovarijetet leptospira.