

*Short communication***OCCURRENCE OF *BARTONELLA HENSELAE*, FeLV AND FIV INFECTION IN 60 STRAY CATS FROM SERBIA**POTKONJAK Aleksandar¹, VRAČAR Vuk^{1*}, STANČIĆ Ivan¹, SPASOJEVIĆ KOSIĆ Ljubica¹, BACIĆ Dragan², CINCOVIĆ Marko¹, TOHOLJ Bojan¹, STEVANČEVIĆ Ognjen¹, RISTIĆ Zoran³

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The aim of this study was to determine the prevalence of coinfection with pathogens *Bartonella henselae*, feline immunodeficiency virus, and feline leukemia virus in stray cats from the area of Novi Sad and Belgrade, Serbia. Each of 60 individual cats was clinically examined and the blood sampled. Therewithal an epidemiological survey was made. Blood sera were separated by centrifugation and serologically tested in order to determine the presence of *Bartonella henselae* specific antibodies (by direct immunofluorescence assay), feline immunodeficiency virus specific antibodies (by rapid test SNAP Combo) and feline leukemia virus antigens (by rapid test SNAP Combo). Of the 60 cat sera, serologically examined using IFA test, 33 (55%) were positive for the presence of IgG specific to *B. henselae* antigens. A total of 13 (27%) of the 60 tested cat sera were positive for the presence of specific antibodies to FIV antigens. None of the 60 tested cat sera were positive for the presence of FeLV antigen. Of the 33 cat sera which contained IgG antibodies to *B. henselae*, 6 cat sera also gave a positive reaction to the presence of specific IgG antibodies to FIV; this was a coinfection seroprevalence of 10% in the total population of studied cats. The results obtained in this study indicate the presence of *B. henselae* and FIV coinfection in cats from Serbia, without FeLV positive cats. An increase in the manifestations of clinical symptoms in cats in which the serological tests determined coinfection with *B. henselae* and FIV is evident compared to those seropositive only to *B. henselae*.

Key words: *Bartonella henselae*, cats, coinfection, feline immunodeficiency virus, feline leukaemia virus

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INTRODUCTION

Bartonella henselae is the causative agent of cat scratch disease (CSD), a disease that occurs in immunocompetent individuals, as well as bacillary angiomatosis and hepatic peliosis as frequent complications in patients with AIDS [1]. Human infection caused by *B. henselae* has all the characteristics of classical zoonosis [2]. Domestic cats are the main reservoir of this pathogen. Seroepidemiological studies indicate a worldwide distribution of infection of cats caused by *B. henselae*. Seroprevalence of antibodies to *B. henselae* antigens, determined in these studies, ranged from 4% to 80%. The prevalence of infection varies depending on the geographic region, from a low prevalence (Norway 0%) in regions with a cold climate to high values of prevalence in regions with a hot and humid climate (Philippines 68%) [3,4]. It was also observed that seroprevalence varies depending on the population of cats, dependent on whether they are stray or pet animals [4].

Due to the high prevalence of *B. henselae* infection in cats, it was difficult to correlate this infection with clinical signs [5]. The clinical course and pathogenesis of bartonellosis in cats differs from the human infection caused by the same pathogen [6]. Immunocompetent and immunosuppressed cats showed a high rate of subclinical infection and remained largely clinically healthy, with signs of chronic bacteremia only [7]. In domestic cats, naturally induced infections can be expressed in the form of gingivitis, stomatitis, lymphadenopathy, uveitis, and urinary tract diseases. On the other hand, experimentally induced infection leads to protracted and repeated, intraerythrocytic bacteremia with the absence of any clinical symptoms, or with the appearance of mild, nonspecific symptoms [6]. Coinfection, with different strains of *B. henselae*, with other species of *Bartonella*, and with other pathogens is possible [8]. A significant increase of the incidence of lymphadenopathy and gingivitis was observed in cats that were coinfecting with *B. henselae* and feline immunodeficiency virus (FIV) [9]. Likewise, a significant correlation between the presence of *B. henselae* antibodies and anti-*Borrelia burgdorferi* antibodies has been reported [10].

The aim of this study was to determine the prevalence of coinfection with pathogens *B. henselae*, FIV and feline leukaemia virus (FeLV) in stray cats from the area of Novi Sad and Belgrade in Serbia.

MATERIALS AND METHODS

Stray cats from the cities of Novi Sad and Belgrade were studied, but for the purpose of this study these cats were treated as one population. Aseptically, 1 mL of whole venous blood from each of 60 individual cats was sampled into vacutainers coated with a clotting factor. At the same time, epidemiological and basic clinical examination of the sampled cats was conducted to determine the presence of lymphadenitis, gingivitis, stomatitis, urological diseases, endocarditis, myocarditis, fever, lethargy, anorexia, local inflammation, neurological and ophthalmological disorders. From each

sample of whole venous blood, after clot retraction in vacutainers, blood serum was separated by centrifugation at 500 x g. Serum samples were frozen at -20° C until diagnostic tests were conducted.

Detection of IgG against *B. henselae* by indirect immunofluorescence assay

The presence of class G antibodies to *B. henselae*, in the blood serum of cats, was determined by indirect immunofluorescence assay (IFA), using Vero cell cultures infected with *B. henselae* and anti-cat IgG sheep serum conjugated with fluorescein isothiocyanate (VMRD Inc., USA). Antibody titres of 1:50 and higher were considered as positive.

Detection of FeLV antigen and FIV antibodies cat sera by SNAP* Combo

To determine the presence of FeLV antigen and antibodies to FIV in the sera of cats, the rapid test SNAP Combo Plus FeLV Ag / FIV Ab (IDEXX Laboratories, USA) was used. Test results were interpreted according to the manufacturer's instructions.

Statistical analysis

Prevalence of clinical signs and statistical significance (CI 95%) of the obtained results were calculated using STATGRAPHICS Centurion XV version 15.2.11 software. Value $p \geq 0.05$ (CI 95%) was considered statistically non significant. For the statistical analysis of the possible effects of FIV infection on the clinical picture of *B. henselae* seropositive cats Odds ratio (OR) was calculated using Open Epi version 3.1 software [11].

RESULTS AND DISCUSSION

Of the 60 cat sera, serologically examined using IFA test, 33 (55%) were positive for the presence of IgG specific to *B. henselae* antigens, while 27 (45%) of tested cat sera were negative (Fig. 1). This seroprevalence value (55%) corresponds to seroprevalence (57%) determined in a previous study conducted in the Republic of Serbia, on a population of 40 cats in the Novi Sad area [12]. In a population of domestic cats in the Netherlands, Bergmans et al. determined a seroprevalence of 56% [13]. Other studies conducted on populations of stray cats in Europe showed lower seroprevalence values. Barnes et al. found seropositivity in 33/79 (41.8%) cats [10]. A seroprevalence value of 39% was reported in Italy [14]. In Austria, Boulouis et al. reported a seroprevalence of 33% [15].

A total of 13 (27%) of the 60 tested cat sera were positive for the presence of specific antibodies to FIV antigens (Fig. 1). FIV antibodies were not found in 47 (73%) of sera of tested cats. FIV seroprevalence worldwide ranges from less than 1% in healthy

cats in North America to 40% in illness-affected cats in Thailand. This range of seroprevalence is caused by geographic location, lifestyle, depending on whether the cats stayed indoors or outdoors and cat clinical status [16-18]. In the first study on the prevalence of FIV in Serbia, 31.6% of the population of stray cats in the Novi Sad area was FIV positive [19]. The reported FIV seroprevalence was 23% in a population of cats from urban areas in Canada [20]. Tozon et al. showed a FIV seroprevalence of 33.3% in a population of cats in Slovenia, where 65% of seropositive cats were stray cats [21]. These data correspond to the results obtained in our study (27%). However, other studies conducted in Europe have shown significantly lower levels of FIV seroprevalence in populations of stray cats. Dorny et al. reported 39 (11.3%) cats positive to the presence of antibodies to FIV in Belgium [22]. Knotek et al., in a seroepidemiological survey conducted on 727 cats in the Czech Republic, reported FIV seroprevalence to be 5.8% [23]. Muirden reported FIV seroprevalence of 10.4% among street cats in the UK [24].

In our study, all of 60 tested cats were seronegative to the presence of FeLV antigen p27 (Fig. 1). Considering the proven presence of this virus in the country and the region [21,25,26], its existence can not be excluded, but the results obtained in our study indicate a low prevalence of FeLV among stray cats in the Republic of Serbia.

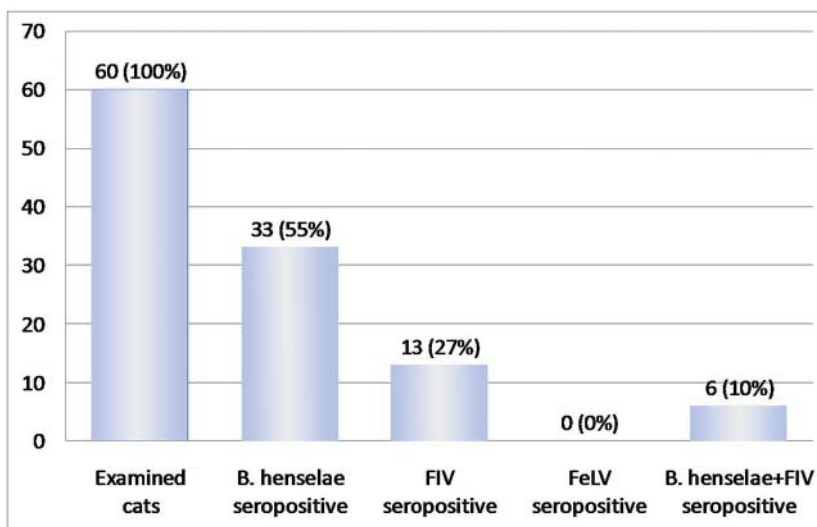


Figure 1. Seroprevalences of infections caused by *B. henselae*, FeLV, FIV and coinfection caused by *B. henselae* and FIV, in 60 stray cats from the area of Novi Sad and Belgrade, Serbia

In the published literature there are few epidemiological studies that have examined the possible correlation between *B. henselae* and FIV or FeLV in cats. To our knowledge this is the first study of its kind in the Republic of Serbia. We found the presence of coinfection caused by pathogens *B. henselae* and FIV in the cat population in the area of Novi Sad and Belgrade, determined by specific IgG antibodies. Of the 33 cat sera

which contained IgG antibodies to *B. henselae*, 6 cat sera also gave a positive reaction to the presence of specific IgG antibodies to FIV; this was a coinfection seroprevalence of 10% in the total population of examined cats (Fig. 1).

Based on the data obtained in this research the prevalence of different clinical signs is calculated, and considering the serological status cats were divided in two groups referring to *B. henselae* seropositive cats and *B. henselae* and FIV seropositive cats. Results of the targeted clinical examination showed that gingivitis (31.7%), lymphadenitis (23.3%), stomatitis (8.3%) and anorexia (6.7%) were the most common conditions found in the examined cats. Of the 6 cats that had a coinfection caused by pathogens *B. henselae* and FIV, 4 (66.7%) cats had gingivitis, while 2 (33.3%) had lymphadenitis, 1 (16.7%) had stomatitis and 2 (33.3%) cats had anorexia. There was no statistically significant difference between the clinical signs prevalences of cats with only *B. henselae* infection, and those with *B. henselae* and FIV coinfection. However, there is a greater risk for the manifestation of clinical symptoms in cats that are coinfecting (gingivitis OR=3.40; lymphadenitis OR=1.75; stomatitis OR=2.50; anorexia OR=2.87) (Table 1). In cats with serological evidence of infection with both pathogens, an increase in the incidence of gingivitis, lymphangitis, stomatitis and anorexia was evident compared to cats with serologically confirmed infections caused only by *B. henselae*. Seroepidemiological studies conducted in Japan showed a significant increase in the incidence of gingivitis and lymphadenopathy in cats with blood sera containing specific antibodies to FIV and *B. henselae* antigens compared to cats which were only *B. henselae* positive [9]. FIV causes a progressive reduction in the number of CD4 + lymphocytes, suggesting that infection caused by *B. henselae* in immunodeficient cats can induce specific clinical signs [27]. In contrast, data from Switzerland indicate that the prevalence or clinical signs of cat infection caused by *B. henselae* were not influenced by FeLV or FIV infection [28].

Table 1. Most common conditions found in the examined cats

Clinical signs	All cats included in the research			<i>B. henselae</i> seropositive cats			<i>B. henselae</i> and FIV seropositive cats			OR
	positive	n	%	positive	n	%	positive	n	%	
Gingivitis	19	60	31.7%	10	27	37.0%	4	6	66.7%	3.40 (0.8-16.0)
Lymphadenitis	14	60	23.3%	6	27	22.2%	2	6	33.3%	1.75 (0.3-12.0)
Stomatitis	5	60	8.3%	2	27	7.4%	1	6	16.7%	2.50 (0.2-33.2)
Anorexia	4	60	6.7%	4	27	14.8%	2	6	33.3%	2.87 (0.4-21.3)

n – number of examined cats; % – prevalence of clinical signs; OR – odds ratio of clinical signs prevalence between *B. henselae* seropositive cats and *B. henselae* and FIV seropositive cats

The results obtained in this study indicate the presence of *B. henselae* and FIV coinfection in cats in Serbia. An increase in the manifestations of clinical symptoms in cats in which the serological tests determined coinfection with *B. henselae* and FIV is evident. Further studies are needed in order to determine the prevalence of FeLV

infection among stray cats in different areas in Serbia, as well as to determine the impact of coinfection of this immunosuppressive virus to infection in cats caused by *B. henselae*.

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POJAVA *BARTONELLA HENSELAE*, FeLV I FIV INFEKCIJE KOD 60 ULIČNIH MAČAKA U SRBIJI

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Cilj ovog istraživanja je da se utvrdi koinfekcija uzročnicima *Bartonella henselae*, virus imunodeficijencije mačaka i virus leukemije mačaka, kod uličnih mačaka s područja Novog

Sada i Beograda, Srbija. Od svake od ukupno 60 jedinki uključenih u istraživanje uzeti su uzorci pune venske krvi. Paralelno s uzimanjem uzoraka obavljen je klinički pregled mačaka i sačinjena je epidemiološka anketa. Krvni serumi izdvojeni su centrifugiranjem i serološki su testirani u cilju utvrđivanja prisustva specifičnih antitela na uzročnike *Bartonella henselae* (testom indirektno imunofluorescencije-IFA) i virus mačije imunodeficijencije (FIV) (brzim testom SNAP Combo), kao i antigena virusa leukemije mačaka (FeLV) (brzim testom SNAP Combo). Od 60 krvnih seruma mačaka, serološki pregledanih korišćenjem IFA testa, 33 (55%) je bilo pozitivno na prisustvo specifičnih IgG antitela na antigene uzročnika *Bartonella henselae*. Od 60 ispitivanih uzoraka seruma mačaka, 13 je dalo pozitivnu reakciju na prisustvo specifičnih antitela na antigene FIV. Niti u jednom od 60 ispitivanih uzoraka seruma nije utvrđeno prisustvo antigena FeLV. Od 33 krvna seruma mačaka, u kojima je ustanovljeno prisustvo specifičnih IgG antitela na *B. henselae*, 6 krvnih seruma dalo je pozitivnu reakciju i na prisustvo specifičnih IgG antitela na uzročnika virusne imunodeficijencije mačaka, što predstavlja seroprevalenciju koinfekcije od 10% u ukupnoj populaciji ispitivanih mačaka. Rezultati dobijeni u ovom istraživanju ukazuju na prisustvo koinfekcije uzročnicima *Bartonella henselae* i FIV kod mačaka u Srbiji, bez FeLV pozitivnih jedinki. Evidentan je porast manifestacije kliničkih simptoma kod mačaka kod kojih je primenom seroloških testova utvrđena koinfekcija izazvana uzročnicima *B. henselae* i FIV, u poređenju sa mačkama kod kojih je ustanovljena infekcija izazvana samo uzročnikom *B. henselae*.