

**FIRST REPORT ON SEROEPIDEMIOLOGICAL AND CLINICAL INVESTIGATION OF CAT INFECTION WITH *BARTONELLA HENSELAE* IN THE AREA OF NOVI SAD, SERBIA**

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*Cat Scratch Disease (CSD) is an infective disease of animals and humans caused with *Bartonella henselae*. Prevalence of infection varies between 0 and 68% depending on different population of cats and geographical region. Naturally infected cats are often clinically healthy and are inapparent germ carriers.*

*In this investigation 40 cats from the area of Novi Sad were analyzed. After the epidemiological questionnaire was made, all cats were clinically investigated. To determine the presence of specific antibodies of class G on *Bartonella henselae* the method of indirect immunofluorescence was used.*

*For the first time in Serbia, in the area of Novi Sad municipality the infection caused by *Bartonella henselae* in the population of cats was detected. Prevalence of specific antibodies of class IgG on *Bartonella henselae* antigen in the population of cats was 57%. The most common clinical manifestations in seropositive cats were gingivitis and lymphadenopathy.*

*Key words: *Bartonella henselae*, cat infection, Cat Scratch Disease, IIF, immunodiagnostics, zoonoses*

#### INTRODUCTION

Infection of cats with *Bartonella henselae* is determined as an infective disease of bacterial etiology, which is most often followed by an inapparent infection. According to the actual classification, the agent of the disease belongs to Genus of *Bartonella* and Family of *Bartonellaceae*. This Family incorporates more than 20 species and subspecies, out of which at least eight represent human pathogens (Boulouis *et al.*, 2005). Cats could be infected except with *Bartonella henselae* also with *Bartonella clarridgeiae*, *Bartonella coehlerae*, *Bartonella quintana* i *Bartonella bovis* (Brunt *et al.*, 2006).

*Bartonella henselae* was isolated for the first time from cats, during the eighties of the last century (Chomel *et al.*, 2006a). Interestingly, presence of DNA

from *Bartonella henselae* genotype Houston was detected in tooth pulp of a cat that lived about 800 years ago. This finding shows that even the agent of the disease was detected recently, the disease is not quite new (La *et al.*, 2004). Domestic cat is main reservoir of *Bartonella henselae*, as well as of *B. clarridgeiae* i *B. koehlerae* (Boulouis *et al.*, 2005). Different types of wild cats also could be infected with Bartonellas (Chomel *et al.*, 2006b). The role of vectors in spreading of different types of Bartonellas is not well known (Breitschwerdt and Kordick, 2000). Presence of cat fleas (*Ctenocephalides felis*) is necessary for the maintenance of infection in the cat population. *Bartonella henselae* reproduced in cat fleas (Finkelstein *et al.*, 2002). Sero-epidemiological and bacteriological studies showed infection with *Bartonella henselae* in domestic cats worldwide (Boulouis *et al.*, 2005). Infections of cats with Bartonellas were registered in different countries of Europe, Asia, Oceania and America (Brunt *et al.*, 2006). Prevalence of infection varies depending of cat populations and geographical region. Values of prevalence of infection are lower in the regions with a cold climate (0% Norway), while in regions with hot and moisture climate are higher (68% Philippines) (Chomel *et al.*, 2006a). Prevalence of antibodies for *Bartonella henselae* antigens varies between 5 and 80%, and prevalence to bacteria varies from few percents up to 50% of cat population (Boulouis *et al.*, 2005).

Establishment of long intracellular bacteriemia in reservoir animals is an important process in the pathogenesis of infection with *Bartonella henselae*. Bartonellas are the only species among bacteria, capable to induce vasoproliferation, beside the known fact that there are some other bacteria that are linked to different tumors. Pathogenesis of vasoproliferation induced by *Bartonella henselae* is complex and includes several biological mechanisms. There are different factors of *Bartonella henselae* virulence that are important in the infection process. The most important are: low toxic LPS, non-fibrillar adhesins (BadA, VompA-D) and Bartonell translocated proteins (BepA-BepG) (Dehio, 2005). Expression of mRNA for IL-4, but no INF- $\gamma$  was observed in infected cats during bacteriemia, which implicates the activation of Th2 immune response (Kabeya *et al.*, 2006). It was also observed that in cats infected with *Bartonella henselae* LSU 16, antibodies of class IgM are detectable three weeks upon infection and that amount of antibodies decreases six weeks upon infection. Maternal antibodies specific for *Bartonella henselae* are transmitted via colostrum on newborns and persist during 10 weeks (Guptill *et al.*, 1998). In some cats infected with *Bartonella henselae* during bacteriemia. A high titer of circulating antibodies was detected. Therefore, the role of humoral immune response in this type of infection is not clear (Freeland *et al.*, 1999). It is reported that the number of bacteria decreases with the concentration of IgG antibodies against *Bartonella henselae*. This humoral immune response significantly improves bactericidal activity. It is considered that antibodies have a certain role in the elimination of Bartonellas, but not enough to eliminate them completely (Kabeya *et al.*, 2006).

Because of high prevalence on infection of cats with *Bartonella henselae* it is quite difficult to link specific clinical symptoms with infection. Cats which are infected experimentally with *Bartonella henselae* show different symptoms like: fever that persist from two days up to few weeks, local inflammation,

lymphadenopathy, lethargy, anorexia, reproductive disorders and neurological problems (Chomel *et al.*, 2004). Also anemia could be present (Breitschwerdt and Kordick, 2000). Naturally infected cats are often clinically healthy and inapparent germ carriers. Seropositive cats often have: lymphadenitis, gingivitis, stomatitis, as well as urological problems (Chomel *et al.*, 2003a). It is believed that *Bartonella henselae* is a potential cause of endocarditis in cats (Chomel *et al.*, 2003b). A link between infection with Bartonellas and uveitis was observed (Ketring *et al.*, 2004). Persistent infection with Bartonellas does not lead to a lethal outcome in chronically infected cats. Clinical symptoms are minimal or not present in persistent infection. Cats infected with *Bartonella henselae* or *Bartonella quintana* do not show pathomorphological changes. Pathohistological changes include peripheral hyperplasia of lymph nodes, follicular hyperplasia of spleen, lymphocytic holangitis/periholangitis, lymphocytic hepatitis, lymphoplasmocytic myocarditis and interstitial lymphocytic nephritis (Kordick *et al.*, 1999). Coinfection with other species and strains of Bartonellas is possible, as well as with other agents of infective disease. A significant correlation between cats seropositive on *Bartonella henselae* and *Borrelia burgdorferi* was described (Shaw *et al.*, 2001). Cats infected with *Bartonella henselae* and Feline immunodeficiency virus (FIV), show increased incidence of lymphoadenopathy and gingivitis, which is explained by immunocompromisation due to CD4+ lymphocyte depletion (Breitschwerdt and Kordick, 2000; Shaw *et al.*, 2001).

Diagnostics based on clinical symptoms is quite difficult due to the fact that many clinically healthy cats are chronically infected (Chomel *et al.*, 2004). Laboratory diagnostics of infection with *Bartonella henselae* include direct and indirect methods. In other words detection of agents from clinical samples and detection of specific antibodies (Rode, 2005). There are many serological detection tests designed for cats. IFA, test use whole bacteria of *Bartonella henselae* as antigen, while others including ELISA use specific single antigens (Brunt *et al.*, 2006). Commercial IFA tests, most often use Vero cell line infected with *Bartonella henselae* (Zbinden *et al.*, 1997). ELISA is still not in use since there is no ideal antigen for this purpose (Rode, 2005). Conducting serological tests is fast and cheap. Negative results of serological tests do not exclude disease, and furthermore there is intercross reaction within Genus Bartonella (Brunt *et al.*, 2006). Intercross reactivity could also be observed with Chlamidia and *Coxiella burnetii* (La Scola and Raoult, 1996; Maurin *et al.*, 1997b). To diagnose human infection with *Bartonella henselae* IFA is standard test (Chomel *et al.*, 2004). There is no study describing specificity and sensitivity of serological tests used for diagnostics of infections with Bartonellas in cats. It is reported that IFA test for presence of IgG antibodies for *Bartonella henselae* is 99% sensitive and 94% specific (Boulouis *et al.*, 2005).

#### MATERIALS AND METHODS

Blood serums from 40 cats from the region of Novi Sad were obtained. Blood sampling was done in veterinarian ambulances and other institutions involved in veterinarian medicine. Each blood sample was followed with an

epidemiological questionnaire and clinical examination of cats. To determine the presence of specific antibodies of class G on *Bartonella henselae* antigens test of indirect immunofluorescence (IIF) was used (manufacturer: VMRD, USA). Test of IIF was made according to manufacturer's instructions. Titer of specific antibodies higher than 1:50 was recognized as positive. Fluorescence intensity of intracellular bacteria ranged between 1+ and 4+.

## RESULTS

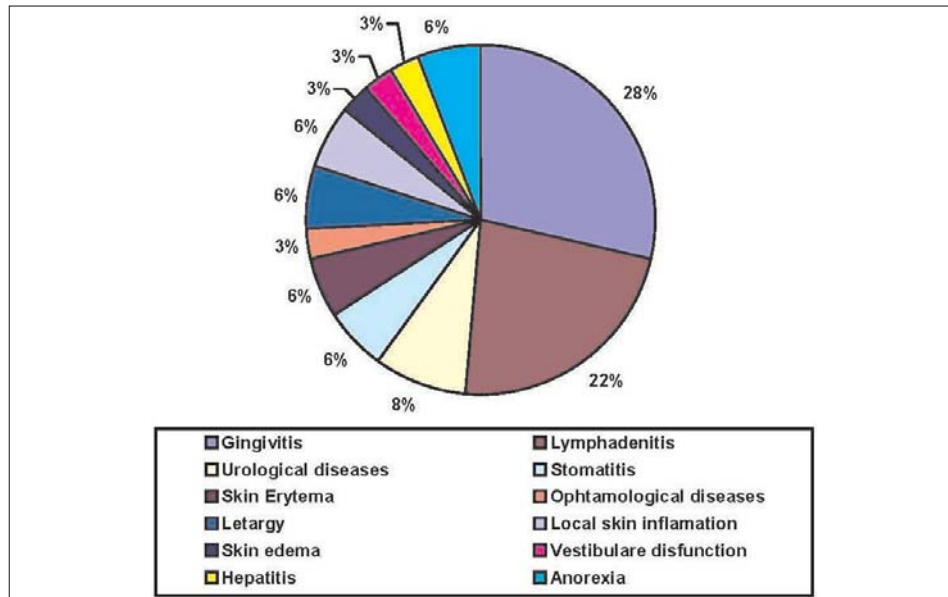
### *Epidemiological questionnaire*

Out of the total number of 27 samples (67%), were collected in veterinary ambulances and other institutions from the field of veterinarian medicine. The samples were collected from cats that were brought for clinical examination, treatment or blood sampling, while 13 samples (33 %) were obtained from healthy cats. In Novi Sad 21 samples were collected (52 %), while 7 samples were obtained from adjacent city zones (18 %). Data about collection area of samples are not known for 12 (30 %) samples. Out of the total number of blood samples, 4 samples (10%) originated from cats that are one year old, 26 (65%) samples are from cats older than one year, while 10 samples (25%) originate from cats for which data are unknown. Males represent 19 (47%) of analyzed cats, females 11 (28%), while data representing the sex of the cats are unknown for 10 samples (25%). Population of analysed cats represents 29 (72%) cats with short hair, 1 (3%) cat with long hair, and 10 (25%) cats for which data are not known. Beside this epidemiological feature we followed the life style of cats. Out of the total number, 12 cats (30%) live in houses together with their owners, while 18 cats (45%) live outdoors. This data are not known for 10 (25%) cats. Predation was a feature of 21 (52 %) cats, while 7 of them were not predators (18%). This data are not known for 12 (30%) cats. Out of the total number 16 (39%) cats were bitten by fleas, while 13 (33%) was not bitten, and data are not known for 11 (28 %) cats. It was found that 28 (70%) cats out of total number were infested by ticks, while this data are not known for 12 (30%) cats.

### *Clinical examination*

Out of 40 cats included in the study, 30 cats (75%) were clinically examined, while 25% were not available. Clinical examination revealed signs and symptoms of disease in 23 (77%) of examined cats, while 7 cats (23%) had no pathological changes. Clinical examination conducted in accordance with the literature data regarding the symptoms of *Bartonella henselae* infection revealed gingivitis as the most frequent change (28%) in tested cats. Lymphadenopathy (22 %) and urological disorders (8 %) were also observed. Sporadically, we observed anorexia (6%), lethargy (6%), local skin inflammation (6%), skin erythema (6%), stomatitis (6%), skin edema (3%), hepatitis (3%), vestibular dysfunction (3%) and ophthalmological changes (3%) (Graph 1). Hematological exam of one cat with observed lymphadenopathy showed leucocytosis ( $11 \times 10^9/L$ ). Body temperature, heart beating and respiration frequency were at physiological range for the species. Clinical exam of cats included in the study did not reveal

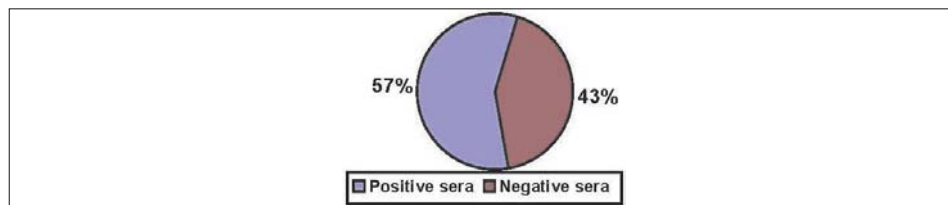
endocarditis, miocarditis, nefritis, attitude alterations or reproductive disorders. Some of observed changes such are phlegmona, otitis externa, conjunctivitis, granulomatous skin proliferation, bronchitis, uterus torsion, opstipation, and skin neoplasia were probably not related to *Bartonella henselae* infection.



Graph 1. Results of clinical examination

*Results of indirect immunofluorescence assay for Bartonella henselae specific IgG antibodies*

Out of 40 serologically examined cats, 17 (43%) were negative on presence of specific antibodies for *Bartonella henselae* (Graph 2). We determined the presence of specific antibodies for *Bartonella henselae* in 23 (57%) blood samples (Graph 2, Fig 1). Antibodies at 1:50 titer were present in 7 sera, 1:100 in 7 sera, 1:200 in 1 serum, 1:400 in 6 sera and 1:800 in 2 sera. Average fluorescence intensity at 1:50 dilution was 3+ arbitrary scale and was detected in 10 sera. The



Graph 2. Results of IIF assay for presence of IgG antibodies against *Bartonella henselae*

majority of cats with a detected presence of specific IgG antibodies against *Bartonella henselae* were males, older than one year, short hair, which live outdoor, with reported predator behavior. The presence of specific antibodies correlated with clinical symptoms of *Bartonella henselae* infection.

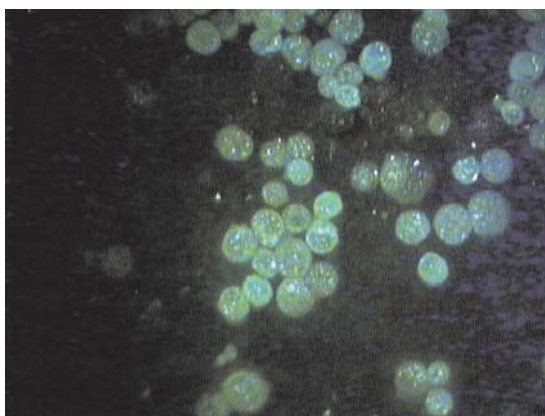


Fig 1. Indirect immunofluorescence, x 400, fluorescence intensity 4+, titer dilution for IgG specific antibodies against *Bartonella henselae* 1:50

#### DISCUSSION

Bartonellas are slow growing, hemotropic, gram negative agents of infective diseases of humans and domestic and wild animals. Infection is persistent and vector borne. Cat scratch disease was described in humans during the fifties of the last century and represents a classical zoonose. Medical importance of this disease is increasing. Cats represent an important reservoir of *Bartonella henselae*. Humans are infected incidentally due to contact with cats, by scratch or by bite (Chomel *et al.*, 2004).

There is no report on the presence and distribution of infection of cats with *Bartonella henselae*. Different factors that lead to infection and distribution of the disease are responsible for the presence of this illness in humans and cats. We investigated the presence of the antigen in the area of Novi Sad. Samples were collected randomly in veterinary ambulances and other institutions of veterinary medicine.

In this study we observed that the group of seropositive cats is dominated by males. Breitschwerdt and associates also demonstrated domination of seropositive males (Breitschwerdt *et al.*, 2005). Maruyama and associates (1988 and 2003) presented similar results. This fact could be explained by the life style of males during estrus of females.

During this period males often cause skin scratches that lead to *Bartonella henselae* infection (Maruyama *et al.*, 1998). Al-Majali did not observe a connection between sex and seropositivity on *Bartonella henselae* (Al-Majali, 2004). Glaus

also described the absence of a correlation between sex and seropositivity on *Bartonella henselae* (Glaus *et al.*, 1997). We determined that seropositive cats are dominantly older than one year. This was also observed by Glaus and Guptill (Glaus *et al.*, 1997; Guptill *et al.*, 2004). Although, most studies show that seroprevalence on *Bartonella henselae* is higher in the population of cats younger than one year (Childs *et al.*, 1994; Chomel *et al.*, 1995; Koehler *et al.*, 1994; Maruyama *et al.*, 2003). Neither Maruyama and associates nor Breitschwerdt had shown the correlation between *Bartonella henselae* seropositivity and cat age (Breitschwerdt *et al.*, 2005; Maruyama *et al.*, 1998). As in this study blood was largely sampled from short haired cats, we cannot draw a conclusion regarding hair length as a risk factor for *Bartonella henselae* infection. Al-Majali determined outdoor life and predator behavior as risk factors for *Bartonella henselae* (Al-Majali, 2004). A similar observation was reported by Breitschwerdt (Breitschwerdt *et al.*, 2005). The result of this study is in line with these findings. Rolain *et al.* (2004a) observed less frequent seropositivity for *Bartonella henselae* antibodies in cats living in house exclusively. It is considered that outdoor cats with predator behavior are more exposed to contact with fleas and other sources of infection. Yet, Guptill did not observe a correlation between cat habitat and *Bartonella henselae* seropositivity (Guptill *et al.*, 2004). High *Bartonella henselae* seroprevalence levels in cats in the moist and warm climate is explained with optimal conditions for arthropod vectors proliferation such as cat fleas. Maruyama *et al.* (2003) point to the correlation between infestation of cats with fleas and *Bartonella henselae* seropositivity. Al-Majali (2004) also reported an increased percent of seropositivity among flea-infested cats. Similar results were described by Guptill (Guptill *et al.*, 2004). We did not observe a clear correlation between the presence of fleas and ticks and *Bartonella henselae* seropositivity, probably because the study was conducted during the cold months when infestation of cats with ectoparasites is reduced (Gurfield *et al.*, 2001).

This study revealed impaired health status of *Bartonella henselae* seropositive cats. Two conditions with the highest frequency were gingivitis and lymphadenopathy. This could be explained either by higher susceptibility of *Bartonella henselae* infected cats for other diseases or by the inability of infected cats to fight *Bartonella henselae* infection. There is also a possibility that clinically observed gingivitis is a consequence of coinfection with other agents of cat infectious disease such as feline syncytial virus (FeSFV). Glaus observed a significant presence of gingivitis and urological diseases in the group of seropositive cats with *Bartonella henselae* (Glaus *et al.*, 1997). Studies made in Switzerland (country with relatively low seroprevalence for *Bartonella henselae*) show that lymphadenopathy is in correlation with the presence of antibodies against *Bartonella henselae*. Studies made in Japan showed correlation between seroprevalence for *Bartonella henselae* and presence of gingivitis and lymphadenopathy. The same type of correlation was observed in North America. Breitschwerdt *et al.* (2005) did not observe a correlation between seroprevalence and development of gingivitis and lymphadenopathy.

We observed a prevalence of IgG antibodies against *Bartonella henselae* of 57% on the territory of Novi Sad municipality. It is already reported that

seroprevalence for antibodies against *Bartonella henselae* varies between 5 and 80% (Boulouis *et al.*, 2005). According to reports from France the percent of seroprevalent cats is 41% (Gurfield *et al.*, 2001). Seroprevalence in cats in the Netherlands is about 56%. (Bergmans *et al.*, 1997). Seroprevalence was reported in 33% of cats in Austria (Boulouis *et al.*, 2005). Seroprevalence in Denmark is 45.6% (Chomel *et al.*, 2002). Very low percentage of seroprevalence was detected in Sweden, 1% (Hjelm *et al.*, 2002), as well as 2.2% (Engvall *et al.*, 2003). In Switzerland seroprevalence was detected in 8.3% of cats (Glaus *et al.*, 1997). In Great Britain seroprevalence was observed in 41.8% (Barnes *et al.*, 2000). In Italy this percent is 38% (Fabbi *et al.*, 2004). In Portugal, seroprevalence was detected in 6.7% cases (Boulouis *et al.*, 2005). All presented data on seroprevalence of antibodies against *Bartonella henselae* in the population of cats shows correspondence with our result obtained for Novi Sad.

In our investigation we reported infection of cats with *Bartonella henselae* using specific IgG antibodies. This suggests a need for further development of optimal diagnostic procedures exploiting techniques such as PCR, ELISA and Western blotting.

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#### PRVI IZVEŠTAJ O RASPROSTRANJENOSTI I KLINIČKOJ SLICI INFEKCIJE MAČAKA SA *BARTONELLA HENSELAE* NA PODRUČJU NOVOG SADA

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#### SADRŽAJ

Bolest mačijeg ogreba (BMO) je infektivno oboljenje životinja i ljudi izazvano sa *Bartonella henselae*. Prevalencija infekcije varira u zavisnosti od populacije mačaka i geografskog regiona i kreće se od 0 do 68%. Prirodno inficirane mačke su često klinički zdrave, inaparentne kliconoše.

U istraživanje je bilo uključeno 40 mačaka sa područja Novog Sada. Nakon uzimanja epidemiološke ankete, sve mačke su klinički pregledane. Za utvrđivanje prisustva specifičnih antitela klase G na antigene *Bartonella henselae* korišćen je test indirektno imunofluorescencije.

Na geografskom području grada Novog Sada i okoline, po prvi put je u Republici Srbiji, utvrđeno prisustvo infekcije izazvane uzročnikom *Bartonella henselae* u populaciji mačaka. Prevalencija specifičnih antitela klase IgG na antigen *Bartonella henselae* u populaciji mačaka iznosila je 57%. Najčešće kliničke manifestacije kod seropozitivnih mačaka bile su gingivitis i limfadenopatija.