Annals of Agricultural and Environmental Medicine 2020, Vol 27, No 3, 356–360
www.aaem.pl

ORIGINAL ARTICLE

Seroprevalence of Antibodies against *Borrelia burgdorferi* s. l. and *Leptospira interrogans* s. l. in Cats in district of Brno and its environs, the Czech Republic

Alena Žákovská[1,A,C-F], Pavel Schánilec[2,A-B,E], František Treml[2,B-C,E], Monika Dušková[1,B,D-F], Carlos Fernando Agudelo[2,E-F]

[1] *Faculty of Science, Masaryk University, Brno, Czech Republic*
[2] *University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Žákovská A, Schánilec P, Treml F, Dušková M, Agudelo CF. Seroprevalence of Antibodies against *Borrelia burgdorferi* s. l. and *Leptospira interrogans* s. l. in Cats in district of Brno and its environs, the Czech Republic. Ann Agric Environ Med. 2020; 27(3): 356–360. doi: 10.26444/aaem/122804

Abstract

Objectives. The aim of this study is to evaluate the seroprevalence of antibodies of *Borrelia burgdorferi* sensu lato (*Bb*sl) and *Leptospira interrogans* sensu lato (*Li*sl) and their possible concurrence in domestic cats living in variable conditions in South Moravia in the district of Brno and its environs. Additional objectives were to discover possible differences in seroprevalence between groups of cats living in different living conditions, and to determine the spectrum of Leptospira serogroups in cats in the same places.

Materials and method. A total of 360 blood sera from domestic cats of 3 different sets were collected during the period 2013–2015. All samples were examined using ELISA for the detection of IgM and IgG antibodies against *Bb*sl, and the microscopic agglutination test (MAT) for the detection of antibodies against 8 serogroups of *Li*sl.

Results. The ELISA method determined 15.8%, 4.8% and 10.3% IgM anti-Borrelia antibodies in the patient group, shelter cats and street cats, respectively. IgG anti-Borrelia antibodies were found in 6.2%, 9.5%, 5.2%, respectively. Antibodies specific for 5 Leptospira serogroups were detected by the use of MAT in 8.8%, 9.5% and 10.3% of cats from the investigated groups. The total positivity of all examined cats for anti-Borrelia antibodies was 18.0% and for anti-Leptospira – 9.2%.

Conclusions. Cats can be infected with both *Bb*sl and *Li*sl. The obtained results are exclusive to the city of Brno and its environs, and are comparable to the limited previous studies. There is a need for further studies of clinical signs of both infections and the possible transmission of Leptospira by ticks.

Key words

feline, Leptospirosis, Lyme borreliosis, ELISA, Microscopic agglutination test

INTRODUCTION

Lyme borreliosis is a multisystemic bacterial human or animal disease caused by *Borrelia burgdorferi* sensu lato (*Bb*sl) and is the most common tick-borne disease in the northern hemisphere. In character, it is a natural focal infection [1, 2, 3]. From the veterinary and medical point of view, 3 genospecies – *Borrelia burdorferi* sensu stricto, *B. garinii* and *B. afzelii* – are of the greatest importance [1, 2]. *B. burgdorferi* circulation in nature is also maintained by animals serving as reservoirs, whereas humans and pets enter the life cycle of the bacteria randomly. Ticks in all stadia may infest cats.

Many arthropods feeding through blood sucking become vectors of infectious diseases in the domestic cat [4]. The main vector of Lyme disease are the ticks *Ixodidae* (particularly in Europe), *Ixodes ricinus* and *Ixodes persulcatus* [1, 2]. Borrelia has been found previously in many other arthropods and insects in the Czech Republic [5, 6, 7], although their importance for transmission is unclear. Due to the free movement of cat in the habitat, the infected ticks have a greater chance to affect the host, especially in spring and autumn when arthropods become active [8, 9]. Cats are most often infected from ticks that have fed on infected rodents during larval development [4]. Cats were also infected after experimental infestation by infected ticks [9]. The reservoir of the disease can include various kinds of wild or domestic vertebrates. Infection has been reported in about 31 kinds of domestic animals, such as dogs, cats, cattle, sheep and horses [1, 2]. Birds are also one of the main reservoirs, especially migratory species that can spread pathogens over large distances [10]. Infected cats may not infect humans directly. They can, however, bring infected ticks home, but it is unlikely that this would be a source of infection for humans [1, 10, 11]. The infection may, however, occur while improperly removing ticks from a cat, when the person comes in direct contact with such a tick [12].

Leptospirosis is a widespread zoonosis, affecting humans, domestic and wildlife animals through the pathogenic spirochaetes *Leptospira interrogans* sensu lato (*Li*sl), genus *Leptospira*. Cats feeding in the wild could be exposed to pathogens through their prey or contact with other animals. Leptospirosis is a zoonosis with dangerous natural foci.

Address for correspondence: Pavel Schánilec, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
E-mail: schanilecp@vfu.cz

Received: 12.02.2020; accepted: 25.05.2020; *first published: 26.06.2020*

The main reservoir for serovar Icterohaemorrhagiae is, for example, the rat, which is closely bound to human society. They are adapted to the pathogen and therefore serious clinical symptoms will not develop. Rats excrete a large amount of bacteria in their urine which can cause a natural outbreak of disease. When the pathogen infects a host that is not adapted to it, it erupts into an infection that can cause severe clinical diseases. Grippotyphosa form another important serogroup whose main reservoir are voles. The focal point of their incidence is primarily agricultural land as well as lawns in housing estates. For serovars from other serogroups whose reservoirs are rodents and insectivores, the bond to the reservoir hosts is equally important. [13, 14] A suitable environment for the survival of Leptospira is still or gently flowing water. For several months they can even survive in moist soil with a neutral or slightly alkaline pH. Leptospira is sensitive to drought, UV rays and pollution. Pathogenic serovars are sensitive to low temperatures. The seasons that are higher in the risk of spreading infection are late summer and autumn, when the reservoir hosts breed and the ideal temperature and pH levels develop in the environment. The disease is widespread in the world but mostly present in hot and humid areas and locations with frequent rainfall or floods [14, 15, 16].

OBJECTIVES

The aim of this work was to evaluate the seroprevalence of antibodies of *Bb*sl and *Li*sl, and their possible concurrence in domestic cats living in variable conditions in the South Moravia district of Brno and its environs. Additional objectives were to discover possible differences in seroprevalence between groups of cats living in different living conditions, and to determine the spectrum of Leptospira serogroups in southern Moravia.

MATERIALS AND METHOD

Characteristics of locality Brno and environs. Brno is a Central European city with 300,000 inhabitants. Two big rivers flow through the town and there is a dam on the outskirts. The prevalence of infected ticks with *Bb*sl ranges from 8–24% [17].

Animals. All examined cats were domestic cats. A total of 360 blood sera from cats of 3 different sets were collected during 2013–2015: Group 1 – veterinary clinical patients (Small Animal Clinic, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic) – the 'patient group'. Group 2 – cats obtained from one shelter (Tibet shelter, Marefy u Bučovic, Czech Republic) – 'shelter cats', and Group 3, urban free-roaming cats admitted for castration to the city shelter (Brno Metropolitan Police, Czech Republic) – 'street cats'. All samples were examined using ELISA for the detection of anti *Bb*sl antibodies, and microscopic agglutination test (MAT) for the detection of anti *Li*sl antibodies.

Description of Groups

Patient group. Cats evaluated or hospitalized for variable signs of diseases at a Small Animal Clinic. Residual serum samples were used from biochemistry examinations. These patients were strictly or partially outdoor cats which originated from the urban area of the city of Brno, and from the suburban and rural environment of Brno and environs up to 35 km.

Shelter cats. Individual animals collected and placed in a shelter. Cats came from the environs of the shelter, within 20 km. None of the cats had a known history, they were healthy and had a minimum time of 28 days in quarantine. This group was characterized by a high concentration of cats living in artificially created groups.

Street cats. Individuals living in the urban environment of the city of Brno, caught and neutered as part of the urban castration programme. This group was characterized by a lower concentration of cats in specific area determined by their territoriality.

Blood sampling. The blood of all cats was collected from the jugular vein or from the cephalic vein. All samples were taken in awake subjects, with the exception of cats in the street group, where the blood was collected under anaesthesia. To obtain serum, the blood was collected into tubes containing clotting acceleration granules. The serum was then recovered by centrifugation (5,000 rpm for 5 min) and frozen at -20 °C until examination.

The presented clinical study was approved by the Ethical Commission of the University of Veterinary and Pharmaceutical Sciences Brno.

Serological examination – ELISA. The whole-cell culture of *Borrelia burgdorferi* sensu lato contained three strains of *B. afzelii* BRZX27 MSLB 8065 and *B. garinii* BRZX 23 MSLB 8064; *B. burgdorferi* sensu stricto (*Bb*ss) WSLB 8014/1 (BIOVETA, a.s., Czech Republic) was used for coating. The negative control, lot 015317, was a pooled sample of 5 cats that have never been immunized by the use of borrelial vaccine or had any contact with borreliae. The positive control, lot 015416, represents a pooled sample of 3 cats. Each one was immunized with one of 3 antigens used simultaneously on ELISA plates. Positive sera of cats were made to test the vaccine for dogs and cats by Borelym 3. The antibodies produced in immunized cats were tested by Bioveta a.s. in house ELISA for post-vaccination sera testing. Conjugate IgM: anti-Cat IgM, HRP (Horseradish peroxidase) conjugated. Conjugate IgG: anti-Cat IgG-Fc, HRP conjugated (BETHYL LABORATORIES, Inc., Tx), FTA sorbent (lyophilized suspension of *Treponema pallidum*) (IMUNA PHARM, a.s., Slovakia), OPD 1,2 diaminobenzen*2HCl (SIGMA-ALDRICH, spol. s r.o., Czech Republic).

A total of 360 sera samples were examined using a modified ELISA method available in commercial sets (TestLine Clinical Diagnostics s.r.o., Czech Rep.) used for the diagnosis of Lyme borreliosis in human medicine, as described in Vostal and Žákovská (2003) [18].

MAT. The MAT is the test most often used for serological diagnosis of leptospirosis. The method is based on mixing test serum to a specific leptospira culture and subsequent evaluation of the degree of agglutination by using dark field microscopy. Samples were considered as positive when 50% or more of visible leptospires appeared to be agglutinated. [13, 14, 15, 16, 19, 20]. In this study, 8 serovars

of *Li*sl: *L. grippotyphosa, L. icterohaemorrhagiae, L. bratislava, L. canicola, L. sejroe, L. sorex jalna, L. pomona and L. pyrogenes,* representing serogroups Grippotyphosa, Icterohaemorrhagiae, Australis, Canicola, Sejroe, Javanica, Pomona and Pyrogenes, respectively, were used. Titers of 1: 100 and higher were considered positive.

Statistical analysis. To determine whether the individual groups differ statistically in the incidence of positive samples, the Fisher exact test was used at the significance level of $p < 0.05$. Cluster analysis was used to discriminate 3 clusters: ELISA positive, borderline and negative IgM and IgG samples. Data showed normal distribution based on the Kolmogorov-Smirnov test.

RESULTS

A total of 360 samples were examined from the 3 groups of cats for the presence of antibodies against *Borrelia burgdorferi* s. l. and *Leptospira interrogans* s. l.

Borrelia burgdorferi s. l. The total positivity of all the examined cats was (65 out of 360; 18.0%). The patient group had the highest number of positive cats (50 out of 260; 19.2%). However, the appearance of total anti-borrelial antibodies was statistically comparable in all groups. Positive IgM antibodies were detected in 41 samples out of 260 (15.8%) in the patient group; 2 out of 42 (4.8%) in shelter cats; and 6 out of 58 (10.3%) in street cats. The prevalence of IgM antibodies of the patient group was significantly higher ($P < 0.05$) than shelter cats. Positive IgG antibodies were 16 out of 260 (6.2%) in the patient group; 4 out of 42 (9.5%) in shelter cats; and 3 out of 58 (5.2%) in street cats. There was no significant difference in the IgG antibodies, while the highest prevalence of IgG antibodies occurred in the shelter cats. There were 7 cats with simultaneous IgM and IgG positivity in the patient group, but no similar cases were found in shelter cats and street cats (Tab. 1).

Leptospira interrogans s. l. The total positivity of all examined cats for anti *Li*sl antibodies was 9.2%; among these, the highest positivity was found in the street cats (10.3%). On the other hand, the presence of anti *Li*sl antibodies was statistically comparable in all groups; patient group – 8.8%, and shelter cats – 9.5%, respectively. Titers found during this study ranged from 1: 200 – 1: 1600. Regarding the serogroup representation, the shelter group showed positivity to Grippotyphosa and Sejroe. In the group of street cats, only antibodies against serogroup Grippotyphosa occurred. The clinical patients group developed antibodies for a wider range of serogroups: Grippotyphosa, Sejroe, Icterohaemorrhagiae, Australis, Canicola (Tab. 1). The most common occurrence of antibodies in cats was against serogroup Grippotyphosa which were detected several times in the highest dilutions – 1:1600. One cat sample even showed antibodies against serogroups Grippotyphosa, Australis and Icterohaemorrhagiae.

The presence of IgM or IgG antibodies against *Bb*sl, as well as antibodies against *Li*sl, was clearly identified in 6 cats (2.3 %) in the patient group. Multi-infection seropositivity, which indicated the presence of all *Bb*sl and *Li*sl examined antibodies, was observed in 2 cats – 0.8% (Tab. 1). The 6 cats were examined: 1 for chronic vomiting, 1 x fatigue, inappetence, 1 x chronic renal failure, 1 x acute onset seizures and blindness, 1 x lameness and swelling of the paw, and 1 x trauma, severe azotaemia and ascites.

DISCUSSION

Epidemiological information concerning feline natural infections with both vector-borne diseases and leptospirosis in Europe is scarce. Each group of feline patients are commonly seen worldwide and have own characteristics. The patient group were cats examined at the authors' veterinary clinic due to various clinical symptoms. Despite the fact that cats of this group were strictly or partially outdoor, they were owned cats and had better veterinary care, which is the reason they were examined more often. The selection of cats for this group was limited by sufficient serum residues for all serological examinations. Cats from the shelter lived together in a large group at one location; however, their original area of extraction often remained unknown. Under these circumstances it was assumed that there were suitable conditions for the transfer of a number of infections. The highest-risk group based on the probability of infection are street cats: they can be, in terms of their lifestyle, infected with various pathogens and they did not receive any veterinary health care.

Cats, as patients in a clinical setting, could be seen either in the framework of preventive examinations, or even more often

Table 1. Results of Bbsl and Lisl positivity

Group /NO.	NO. IgM anti-Bbsl/%	NO. IgG anti-Bbsl/%	Total of anti-IgM and IgG/%	Total NO. of infection Bbsl/%	NO. Ig anti-Lisl/%	NO. of samples specific for serogroups	Anti IgM or IgG Bbsl and anti-Lisl/%	Anti- IgM and IgG Bbsl and anti- Lisl/%
Patients /260	41/15.8*	16/6.2	7/2.7	50/19.2	23/8.8	13 Lg 4 La 3 Ls 2 Li 1 Lc	6/2.3	2/0.8
Shelter cats /42	2/4.8*	4/9.5	0	6/14.3	4/9.5	3 Lg 1 Ls	0	0
Street cats /58	6/10.3	3/5.2	0	9/15.8	6/10.3	6 Lg	0	0
Total /360	49/13.6	23/6.4	7/2.7	65/18.0+	33/9.2+		6/2.3	2/0.8

Statistically significant (0.05).
* patients against shelter cats; + Bbsl against Lisl; anti Lisl – antibody against Leptospira interrogans sensu lato; anti Bbsl – antibody against Borrelia burgdorferi sensu lato.
Serogroups: Grippotyphosa (Lg); Sejroe (Ls); Icterohaemorrhagiae (Li); Australis (La); Canicola (Lc)

because of diagnosis and treatment of pathological conditions. When an owned cat patient goes outdoors, they may be caught in the life cycle of ticks [1, 2, 9]. This may explain why cats in the clinical patients group showed a statistically significant higher level of anti *Bb*sl IgM antibodies (15.8%; $p < 0.05$) than shelter cats (4.8%). This higher incidence of antibodies might be explained, at least partially, by the fact that when cats were taken for certain health reasons to a veterinary clinic, they could have been already in an early phase of infection; that is the time when this group of antibodies form and the animal shows non-specific reaction. Interestingly, 7 cats from the patient group had simultaneous positivity for *Bb*sl IgM and IgG (2.7%), a situation that did not occur in the other groups. Patients in the acute or late-acute phase of disease may show IgM elevations. Though there is a wide range of presentation of clinical signs, actually more notorious and prolonged signs are noticed in the middle-to-late course of the disease like lameness, neurological signs, renal involvement, etc. that may be or not accompanied also by acute elevations of IgM [1, 2, 9]. The shelter cats showed the lowest incidence of anti-Borrelia IgM (4.8%). These antibodies are formed in the early phase of infection; therefore, their low presence corresponded with the fact that living in a shelter resulted in a very small probability of being infected by a tick. The cats that were found to be infected could have been placed in the shelter in the first weeks of infection when these antibodies began to form. However, it is still not known whether other risk factors could also influence antibody reactions, e.g. the reason for being left in the shelter, past risks of exposure, and the length of time living at the shelter. Unlike the very low number of IgM positive cats, the IgG (9.5%) in this group was higher than in other groups, but not statistically significant. The IgG antibodies are formed later and their level could persist for many months [4]. Both findings (low number of IgM and higher number of IgG) could be also explained by the fact that all shelter cats had a minimum of 28 days quarantine.

Cats can be infected only through a vector (tick) but not directly among themselves [1, 2, 4]. It is most likely, in the case of shelter cats with an increased level of IgG antibodies, that prior to their placement in the shelter they had been found in an environment with a high risk of tick infestation, thus being infected with borreliosis [1, 2, 4, 9].

The third set of examined cats was caught in the streets of the city of Brno. Their positivity for anti-*Bb*sl IgM antibodies reached 10.3% and for IgG – 5.2%. The occurrence of ticks in cities also increases [4, 9, 17, 21] which also brings a higher risk for spreading infectious tick-borne diseases, as found in the group of street cats.

Leptospirosis is transmitted by direct contact with infected animals or their urine, through an injury, or by hunting and/or ingesting a reservoir host, behaviour which is very common for cats [13, 14, 15, 16]. All secretions and excretions of infected animals can be a source of infection [22]. Transmission is also possible via the placenta, venereal routes or milk during lactation. Although there is evidence of spirochetes temporarily surviving in insects and other invertebrates, the arthropod's role as a vector has yet to be completely understood [23]. Indirect infection occurs most often when drinking from puddles contaminated with the urine of infected rodents, and also from contaminated soil, food or vegetation [13, 14, 15, 16]. Regarding risks for cat owners, there has not yet been any documentation of infection spread from a cat to a human being [11, 14].

Within the *Li*sl complex, until now there are over 200 antigenically different serovars belonging to more than 20 serogroups [13, 14]. Leptopspirosis in cats and dogs is usually caused by serovars of serogroups Icterohaemorrhagiae, Pomona, Grippotyphosa, Ballum and Canicola [13, 14, 24, 25, 26]. Other authors [27, 28] additionally define the serovar *L. bataviae*. The current study confirmed the occurrence of antibodies against 5 Leptospira serogroups, Grippotyphosa, Icterohaemorrhagiae, Australis, Canicola and Sejroe. Moreover, the results obtained show antibodies against the serogroup Australis, compared with previous studies.

A rate of 10.3% was the highest reported incidence of Leptospira antibodies in the street cats. These cats were considered to have a very high occurrence of antibodies due to their non-domestic way of life, despite not living in a humid or flood area. They spend a considerable part of their lives outdoors, therefore are very likely to come in contact with infected ticks and rodents. Therefore, neither source of infection can be excluded [23]. Rodents are closely related to human society, therefore their presence in large cities is no exception [13, 14]. On the other hand, the highest individual number of samples that were positive for antibodies to Leptospira was found in the patient group. This fact was expected because cats as predators hunt mostly rodents, which are reservoirs of leptospirosis. Since this disease is transmitted by direct contact with infected animals, contact while catching and then ingesting the infected rodent may represent the highest risk of infection [13, 14, 15, 16]. In addition, cats living both fully and/or partially outdoors can come in contact with the urine of infected wild animals, or infected dogs and cats in their neighbourhood [16]. Furthermore, owned cats have better veterinary care if they show any clinical problems.

The incidence of antibodies to Leptospira in shelter cats was 9.5%. Since leptospirosis is spread through direct contact with the urine of an infected animal, theoretically just one infected animal in the group could infect other individuals or could be infected before being located to the shelter [16].

A limitation in this study is the fact that studies concerning the incidence of anti *Bb*sl and *Li*sl antibodies in cats are scarce, which may have broadened the interpretation of the presented results unless future studies will be published. In general, feline Lyme disease is mentioned more often than feline leptospirosis in most parts of the world. One study described the presence of antibodies against *Borrelia burgdorferi* sensu stricto in naturally infected cats in Connecticut, USA. In the endemic areas of north-eastern USA, a seroprevalence ranging from 47–71% was found [29, 30]. In Europe, it was reported that the prevalence of *Bb*sl antibodies in cats ranges 0–37% [31]. In the presented sample of 360 cats, the positivity of borrelia antibodies was 13.6% for IgM and 6.4% for IgG. These determined values were not higher than the values defined by other authors, and are in the middle range of European defined values.

Both naturally and experimentally induced Lyme disease were described in cats [1, 2, 4, 32]. Shaw and Day [4] stated that experimentally infected cats had lymphocytosis and eosinophilia. In a recent clinical study on 30 naturally infected cats, musculoskeletal manifestations were observed in 24%, another 22% suffered from anorexia, fever or fatigue [29]. The latter is supported by another study which reported that cats can develop lameness, fever or anorexia [33]. However, these clinical signs cannot be clearly associated with this disease [8, 29]; on the contrary, many authors state that borreliosis

in cats occurs without any clinical symptoms [2, 4, 32]. It was found that seroprevalence ranged from 8.8% – 33.3%, depending on the season [34].

Regarding feline leptospirosis, Larsson [35] reports positive results in 12.8% of the studied population by using the MAT method. Increased levels were detected in adult cats, which is logical since age increases the risk of contact with infectious agents [1]. In Scotland, a seroprevalence of 9.2% was found in cats [36]. Other authors state that the presence of *Li*sl antibodies ranges between 2–25% [1]. More recent publications state seroprevalence ranging from 7.2–14.9% [8]. In the current study, the average positivity was 9.2% to Leptospira in all the studied groups. The 6 cats of the patient group showed positive results for both infections, but it was not possible to decide whether any of the clinical signs were caused by these infections.

The aim of this work was to contribute to a wider knowledge about Lyme disease and leptospirosis infections in the domestic cat, especially in terms of their incidence in populations closely related with human due to their zoonotic potential. The detected occurrence of antibodies against *Bb*sl and *Li*sl is valid for the area of Brno and its environs and is the only such study in the Czech Republic. The obtained results did not exceed the rarely reported values in the literature. Antibodies against Lyme disease are more common than Leptospira antibodies. Serogroup Australis seems to be one of the possible causes of cat infection.

CONCLUSIONS

Cats can be infected with both *Bb*sl and *Li*sl. The obtained results are comparable with the limited previous studies and are exlusive to area the city of Brno and its environs. There is a lack of awareness of the possible clinical manifestations of these diseases in cats. Further studies should pay attention to maintain the monitoring of possible clinical signs of positive cats and the possible concurrence of both infections. Cats can be also used as sentinel animals for monitoring space risk for humans. Attention must also be given to possible transmission of Leptospira by ticks.

REFERENCES

1. Greene CE, Straubinger RK, Levy SA. Borreliosis. In: Greene CE. Infectious diseases of the dog and cat. 4rd ed. St. Louis: Elsevier Saunders; 2012. p. 447–465.
2. Sykes JE. Lyme Borreliosis. In: Sykes JE. Canine and feline infectious diseases. St. Louis: Elsevier Saunders; 2014. p. 487–497.
3. Aberer E. Lyme borreliosis-an update. J Dtsch Dermatol Ges. 2007; 5: 406–414.
4. Hovius EK. Borreliosis. In: Shaw SE, Day MJ. Arthropod-borne infectious diseases of the dog and cat. London: Manson Publishing, 2005. p. 100–109.
5. Netušil J, Žákovská A, Vostal K, Norek A, Stanko M. The occurrence of Borrelia burgdorferi sensu lato in certain ectoparasites (Mesostigmata, Siphonaptera) of Apodemus flavicollis and Myodes glareolus in chosen localities in the Czech Republic. Acta Parasitol. 2013; 58: 337–341.
6. Hubálek Z, Halouzka J, Juřicová Z. Investigation of haematophagous arthropods for borreliae – summarized data 1988–1996. Folia Parasitol. 1998; 45: 67–72.
7. Netušil J, Žákovská A, Vostal K, Norek A, Stanko M. The occurrence of Borrelia burgdorferi sensu lato in certain ectoparasites (Mesostigmata, Siphonaptera) of Apodemus flavicollis and Myodes glareolus in chosen localities in the Czech Republic. Acta Parasitol. 2013; 58(3): 337–41.
8. Rodriguez J, Blais MC, Lapointe C, Arsenault J, Carioto L, Harel J. Serologic and Urinary PCR Survey of Leptospirosis in Healthy Cats and in Cats with Kidney Disease. J Vet Intern Med. 2014; 28: 284–293.
9. Littman MP, Gerber B, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med. 2018; 32: 887–903. https://doi.org/10.1111/jvim.15085.
10. Birchard SJ, Sherding RG. Saunders manual of small animal practise. 3rd ed. St. Louis, Missouri: Elsevier, 2006.
11. Brown RR, Elston TH, Evans L, Glaser C, Gulledge ML, Lappin MR, Marcus LC. Feline zoonoses guidelines from the American Association of Feline Practitioners. J Feline Med Surg. 2005; 7: 243–274.
12. Rabinowitz PM, Gordon Z, Odofin L. Pet-related infections. Am Fam Physician. 2007; 76: 1314–1322.
13. Greene CE, Sykes JE, Moore GE, Goldstein RE, Schultz RD. Leptospirosis. In: Greene CE. Infectious diseases of the dog and cat. 4rd ed. St. Louis: Elsevier Saunders; 2012. p. 431–447.
14. Sykes JE. Leptospirosis. In: Sykes JE. Canine and feline infectious diseases. St. Louis: Elsevier Saunders; 2014. p. 474–486.
15. Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE. 2010 ACVIM Small Animal Consensus Statement on Leptospirosis: Diagnoses, Epidemiology, Treatment, and Prevention. J Vet Intern Med. 2011; 25: 1–13.
16. Schuller S, Francey T, Hartmann K, Hugonnard M, Kohn B, Nally JE, et al. European consensus statement on leptospirosis in dogs and cats. J Small Anim Pract. 2015; 56: 159–179.
17. Žákovská A, Nejezchlebová H, Bartoňková N, Rašovská T, Kučerová H, Norek A, Ovesná P. Activity of the tick Ixodes ricinus monitored in a suburban park in Brno, Czech Republic, in association with evaluation of selected repellents. Journal of Vector Ecology 2013; 38(2): 295–300.
18. Vostal K, Žákovská A. Two-year study of Examination of Blood from Wild-living Rodents for the Presence of Antiborrelian Antibodies. Ann Agric Environ Med. 2003; 10: 1–4.
19. Shropshire SB, Veir JK, Morris AK, Lappin MR. Evaluation of Leptospira species microscopic agglutination test in experimentally vaccinated cats and Leptospira species seropositivity in aged azotemic client-owned cats. J Feline Med Surg. 2016; 18(10): 768–772.
20. Terpstra WJ. Human leptospirosis: Guidance for diagnosis, surveillance and control. World Health Organisation, 2003.
21. Žákovská A, Nejezchlebová H, Bartoňková N, Rašovská T, Kučerová H, Norek A, et al. Activity of the tick Ixodes ricinus monitored in a suburban park in Brno, Czech Republic, in association with evaluation of selected repellents. J Vector Ecol. 2013; 38: 1–6.
22. Fox JG, Anderson LC, Loew FM, Quimby F. Laboratory animal medicine. 2nd ed. London: Elsevier; 2002.
23. Wójcik-Fatla A, Zając V, Cisak E, Sroka J, Sawczyn A, Dutkiewicz J. Leptospirosis as a tick-borne disease? Detection of Leptospira spp. in Ixodes ricinus ticks in eastern Poland. Ann Agric Environ Med. 2012; 19(4): 656–659.
24. Azócar-Aedo L, Smits HL, Monti G. Leptospirosis in dogs and cats: epidemiology, clinical disease, zoonotic implications and prevention. Arch Med Vet. 2014; 46: 337–348.
25. Sessions J, Greene C. Canine leptospirosis: epidemiology, pathogenesis and diagnosis. Comp Cont Educ Pract Vet. 2004; 26: 606–618.
26. Euzéby, JP. List of Bacterial Names with Standing in Nomenclature: a Folder Available on the Internet. Int J Syst Bacteriol. 1997; 47: 590–592. doi:10.1099/00207713-47-2-590
27. Lappin MR. Polysystemic Bacterial Diseases: Leptospirosis. In: Nelson RW, Couto CG, Small Animal Internal Medicine. 4rd ed. St. Louis, Missouri: Mosby; 2009. p. 1315–1317.
28. Songer SG, Post KW. Veterinary microbiology: Bacterial and fungal agents of animal disease. St. Louis, Missouri: Saunders Elsevier; 2005.
29. Magnarelli LA, Bushmich SL, Ijdo JW, Fikrig E. Seroprevalence of antibodies against Borrelia burgdorferi and Anaplasma phagocytophilum in cats. Am J Vet Res. 2005; 66: 1895–1899.
30. Levy SA, O'Connor TP, Hanscom JL, et al. Evaluation of a canine C6 ELISA Lyme disease test for the determination of the infection status of cats naturally exposed to Borrelia burgdorferi. Vet Ther. 2003; 4: 172–177.
31. Pantchev N, Vrhovec MG, Pluta S, Straubinger RK. Seropositivity of Borrelia burgdorferi in a cohort of symptomatic cats from Europe based on a C6-peptide assay with discussion of implications in disease aetiology. Berl Munch Tierarztl Wochenschr. 2016; 129(7–8): 333–339.
32. Burgess EC. Experimentally induced infection of cats with Borrelia burgdorferi. Am J Vet Res. 1992; 53: 1507–1511.
33. Colville JL, Berryhill DL. Handbook of zoonoses: identification and prevention. St. Louis, Missouri: Mosby; 2007.
34. Magnarelli LA, Anderson JF, Levine HR, et al. Tick parasitism and antibodies to Borrelia burgdorferi in cats. J Am Vet Med Assoc. 1990; 197: 63–66.
35. Larsson CE, Santa Rosa CA, Hagiwara MK, Paim GV, Guerra JL. Prevalence of feline leptospirosis: serologic survey and attempts of isolation and demonstration of the agent. Int J Zoonoses. 1984; 11: 161–169.
36. Agunloye CA, Nash AS. Investigation of possible leptospiral infection in cats in Scotland. J Small Anim Pract. 1996; 37: 126–129.