

CANINE LEISHMANIOSIS (*LEISHMANIA INFANTUM*) IN SLOVENIA: A QUESTIONNAIRE-BASED SURVEY

Tina Kotnik^{1*}, Katja Ahačič¹, Ana Rostaher², Patrick Bourdeau³

¹Clinic for Surgery and Small Animal Medicine, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; ²Clinique Vétérinaire Advetia, 5 rue Dubrunfaut, 75012 Paris; ³Ecole Veterinaire de Nantes, Bp40706, Nantes Cedex 03 44307, France

*Corresponding author, E-mail: tina.kotnik@vf.uni-lj.si

Summary: Leishmaniosis is a disease caused by different species of a protozoan parasite of the genus *Leishmania* but the most common and broadly studied in dogs is the infection with the species *Leishmania infantum*, which is also transmissible to humans. The primary objective of this study was to obtain an epidemiological estimate on canine leishmaniosis in Slovenia during the period from 2005 to 2010. Questionnaires were sent to 105 slovenian veterinary practices, 49 responded and amongst them, 42 (85,7 %) didn't diagnose a case of leishmaniosis in a dog in the past five years. Slovenian veterinarians observed, that the canine leishmaniosis case numbers were descending or at least stagnating during the estimated period. All dogs, diagnosed to have leishmaniosis, were imported from endemic regions, mostly from Spain and France and occasionally from Portugal, Italy, Croatia and Africa. Constantly to frequently skin lesions, such as alopecia, exfoliative dermatitis, ulcers, nodules, pyoderma, lesions on the bridge of the nose (depigmentation, ulcers) and lesions on footpads (excessive nail growth, hyperkeratosis, ulcers) were observed. Eye and eyelid lesions like periocular alopecia, nodules at the edge of the eyelid, conjunctivitis and uveitis and beside these, non-dermatological signs such as apathy, elevated body temperature, anaemia, weight loss and diarrhea were frequently observed. One third of Slovenian veterinary practices had euthanized their cases in spite of the treatment and they have decided for euthanasia most often because of chronic renal failure (CRF) and zoonotic potential. Results of this survey showed that at least until 2010, Slovenia cannot be considered as an enzootic area. However an increase of travelling Slovenian dogs to the other Mediterranean countries may result in an increase of the probability of the diagnosis on imported cases. Utilization of diagnostic tests such as IFAT and PCR on a broad population of healthy and diseased dogs, together with vector analysis is proposed for the future studies.

Key words: leishmaniosis; dogs; *Leishmania infantum*; zoonosis; sand fly

Introduction

Leishmaniosis is a disease caused by different species of a protozoan parasite of the genus *Leishmania* (1). This genus includes approximately 30 species of which 20 are pathogenic to humans (2) and at least 10 to dogs (3). In dogs, the most common and broadly studied is the infection with the species *Leishmania infantum* (2), which is also transmissible to humans (4). The transmission is due to infective bites of sandflies (dipterans phle-

botiminae). For each species of *Leishmania* only a limited number of species of these insects are able to ensure the development of the parasite and the transmission. Dogs are considered as the main reservoir of this infection for humans (5). Infection with this species commonly produces the most severe form of the disease in dogs as well as in people, so called visceral form, which is often fatal, especially if left untreated (6, 7). Visceral form of human leishmaniosis caused by *L. infantum* has been continuously present in Spain, Greece, Italy, Portugal and south of France with about 300 - 400 new human cases annually reported in the southern regions of Europe (4,8). Climate changes

in the last decade could have enabled the formation of new endemic regions throughout Europe that are located north of the existing ones (9,10).

The lesions may concern virtually every tissue but most common clinical signs in dogs include skin lesions (11), swollen lymph nodes (12) and lesions in the eyes (13), and the most common cause of death is kidney failure (14). The diversity of clinical signs has been recently reviewed (15).

A diagnosis is made by parasitological, serological and molecular tests (16). Cytological examination of damaged and altered tissue and the extraction of pathogen's DNA from these tissues with polymerase chain reaction (PCR) are two of the most reliable among the methods detecting the parasite (17). Quantitative serology combined to a complete clinical and biochemical panel is the most relevant technique for an accurate prognosis and the choice of the best therapeutic approach (5).

Current treatment protocols in veterinary medicine include meglumine antimoniate or miltefosine, combined to allopurinol (7). These drugs can be used to improve or control the clinical condition of the dog, but do not eliminate infection (18).

Prevention of disease transmission is based mostly on the reduction of risk of exposure to insect bites (areas, period of the year, period of the day) but mainly on the use of repellents and insecticides with demonstrated preventive efficacy (pyrethroids) (19); however a lot of funding and research has recently been put into developing a vaccine (20). A vaccine is now available in some European countries.

The primary objective of this study was to obtain an epidemiological estimate on canine leishmaniosis in Slovenia during the period from 2005 to 2010. Slovenia is small EU country located below the Alps but sharing the coast of the Adriatic Sea with Italy and Croatia. Since both neighbouring countries belong to the endemic area (9, 21) there is the reasonable concern about Slovenia. We hypothesized that Slovenia haven't belong to endemic region. Climate at Slovenian coast has traditionally been sub Mediterranean, while majority of the state has been sharing continental and alpine climate. Although the temperatures associated to these climates do not seem favourable, an extension of potential vectors has been already observed in Italy both in the northeast and in the north through alpine valleys toward close to Switzerland (9). We hypothesized that veterinarians in

Slovenia have already had diagnosed leishmaniosis in dogs, imported from endemic areas.

Material and methods

Questionnaires

Veterinary practices in Slovenia with at least partial practice in small animal medicine (n = 105) were included into the study. Each of them received one questionnaire designed to be filled by one practice. Confidentiality of collected data was provided. The study lasted from beginning of May 2010 until the end of October 2010. The data were collected on dogs only, using a questionnaire, which had already been used in other European countries doing the same epidemiologic research (France, Portugal, Greece, Spain, Italy) under the guidance of one of the authors (P.B). Questionnaire was translated to Slovene language and adapted with slight modifications and consisted of 8 sections:

1. general data on veterinary practice
2. importance of leishmaniosis for the practice
3. clinical signs
4. diagnosis
5. treatment
6. follow up
7. prognosis
8. prophylaxis
9. public health

Even the vets, who had never diagnosed a single case, were able (and were asked) to give answers to sections, numbered 1, 8 and 9. This way, we were able to collect more data.

The course of the events

Questionnaires were sent to the practices by post at the beginning of May 2010. An invitation letter and prepaid envelope were added to the questionnaire. The address on the envelope was of Veterinary faculty of Ljubljana where returned questionnaires were collected. Confidentiality of collected data was provided. By the end of June 2010 a phone survey was combined to increase the number of answers. We found out that many vets didn't understand they were welcome to participate to the study even if never diagnosed a case

resulting in a loss of questionnaires at this point. By the request they were sent again via mail or E-mail. Returned questionnaires had been analyzed each time and responses of the practices had been evaluated.

In September 2010 a third call and sending of the questionnaires to the practices was made and at the end of October 2010 questioning was closed by receipt of 49 questionnaires.

Results

Questionnaires were sent to 105 veterinary practices in Slovenia with 49 (46,7 %) responders. Amongst the latter, 42 (85,7 %) didn't diagnose a case of leishmaniosis in a dog in the past five years.

In seven Slovenian veterinary practices veterinarians diagnosed at least one CanL case in the period between 2005 and 2010 and estimated that the number of cases was staying the same (50 %) or getting lower (50 %). All responders shared the opinion of not being practicing in an enzootic area (not having diagnosed autochthonous cases) since they diagnosed leishmaniosis only in dogs which have been imported mostly from Spain and France or occasionally from Portugal, Italy, Croatia and Africa.

The clinical signs observed are indicated in table 1.

Slovenian veterinarians most frequently suspected leishmaniosis according to the results of blood tests as CBC and biochemistry profile. Less of the practices have used urinalysis. One prac-

Table 1: Symptoms of dogs with leishmaniosis, diagnosed in Slovenia in the period between 2005 and 2010 (number indicates the number of clinical practices)

Frequency of observation / symptom	Never	Seldom	Periodically	Frequently	Very frequently	Constantly
Apathy, lethargy		1		3	1	2
Fever		1	1	2	1	1
Anemia			1	1	2	1
Weight Loss			1	3	2	2
Alopecia			3	2		
Exfoliative dermatitis with large scales		1	2	3	1	
Exfoliative dermatitis with small scales			2	2		
Skin ulcers		1	1		1	1
Pyoderma			3	2		
Skin depigmentation		2	4			
Skin nodules		2	2	1	1	
Onychogryphosis			3		1	
Nasal depigmentation, ulceration		1	3	1		
Footpads depigmentation, ulceration			3		1	1
Ocular lesions		1	1		2	1
epistaxis		2	1		1	
Diarrhea			3	1	2	1
Lymphadenopathy			1	1	3	
Renal failure			2	2	1	
Polyarthritis			4			
Osteomyelitis		1	3			

Comments to table 1: Every number in the table represents answer of one veterinary practice. Concurrently to leishmaniosis, anaplasmosis has been diagnosed by one veterinary practice in two *Leishmania*-infected (CanL) dogs.

tice has frequently used protein electrophoresis. Antinuclear antibody test has been periodically used by one practice while Coombs test has never been used amongst nonspecific tests.

Slovenian veterinarians have used enzyme-linked immunosorbent assay (ELISA) for *Leishmania* antibody titer determination most often to confirm the diagnosis. Two practices have used rapid tests (SNAP® Leishmania Test; IDEXX Laboratories) and PCR and one has used histopathology, immunofluorescent-antibody test (IFAT) or lymph node biopsy to confirm the diagnosis. Majority of the practices have never used fine needle biopsy of the skin, lymph nodes, bone marrow or spleen to provide materials for cytology. Slovenian veterinary practices have sent materials for specific tests to the next specialized laboratories: Institute for microbiology and parasitology of Veterinary faculty of Ljubljana (50 %), Human national laboratory (10 %) and private laboratories like Laboklin (Austria) and Invitro (Austria).

Therapy

A list of drugs was proposed in the questionnaire and their use indicated in the table 2

Majority of the practices have used allopurinol as a single agent to treat canine leishmaniosis. Glucantime has periodically been used only by one practice and miltefosine was not utilized (this molecule was not available on the veterinary market before 2007 and was launched only in some European countries not including Slovenia).

Several other medicaments, like quinolones and azoles have been used by certain practices on regular basis. Occasionally pain at application site has been observed with antimoniate treatment and the appearance of crystals in the urine has been noticed with allopurinol treatment. Reported side effects haven't led to drug discontinuation.

Half of the practices that have treated CanL stopped their treatment on the basis of clinical improvement and a quarter of practices on the basis of protein analysis. For the rest, criteria stood unknown. In most of the cases treatment was reinstated due to clinical relapse and in some cases on the basis of equal or higher antibody titer at the control exam. Rarely, veterinarians decided to start the treatment again on the basis of skin and lymph node cytology. Control exams were usually performed yearly. Half of the practices lost their patients for follow up and survival time of *Leishmania*-infected dogs was estimated to be 2-5 years.

Half of the practices periodically suggested euthanasia, the rest rarely or never. Nevertheless, 1/3 of *Leishmania*-infected dogs were euthanized anyway.

Prevention

Answers to the prophylactic section were given by 49 out of 105 veterinary practices. Most of them (92,3 %) have advised the use of insecticides and repellents to prevent spread of leishmaniosis. Beside the use of insecticides and repellents, we can see that Slovenian veterinarians have most fre-

Table 2: Drugs that have been used by Slovenian veterinary practices in the treatment of dogs with leishmaniosis in the period from 2005 to 2010 (Every number in the table represents answer of one veterinary practice).

	Never	Periodically	Frequently	Constantly
Glucantime (antimoniate)		1		
Pentostam (antimonat)				
Pentamidin				
Amphotericin B				
Miltefosin				
Allopurinol				3
Ketoconazol				1
Metronidazol				1
Enrofloxacin		1		2
Marbofloxacin				1

Table 3: Reasons why Slovenian veterinarians decided for euthanasia of dogs with leishmaniosis (period 2005 to 2010)

REASON FOR EUTHANASIA	Frequency			
	Never	Periodically	Frequently	Constantly
Children in house	1	1	2	
Elderly people in the house	1		1	
People who are receiving immunosuppressive therapy	1		1	
HIV positive people	1			
Other dogs in the house			1	
Live in an endemic area	1			
Medical expenses	2	1		
The owner refuses treatment	1	1	1	
Kidney failure	1	1	1	2
Eye lesions	1			
Other causes (specify): -zoonotic risk			1	

Comments to table 3: Every number in the table represents answer of one veterinary practice.

quently suggested staying in the house during the night and the use of nets on the windows (Table 4). Seventy-two percent of the practices have advised the use of insecticides and repellents when the dog travelled to an enzootic area even if the owner did not show interest for prevention. Almost half of the practices (48,5 %) instructed the use of permethrin and imidaclopride spot-on combination

(Advantix[®], Bayer), one third instructed the use of deltamethrin collars (Scalibor[®], Intervet), and the rest proposed permethrin spot-on (Ex Spot[®], Intervet) utilization. One third of the owners (31,3 %) wanted to get instructions on the preventive measures when they were about to move to the endemic area and less of them (22,9 %) if they had already been living in the endemic area.

Table 4: Preventive measures suggested from Slovenian veterinarians (period 2005 to 2010) (each number represents answer of one veterinary practice)

The patient should stay in the house at night	Window nets	Use of repellent insecticides	Use of insect traps (example: electric trap)	Avoiding contact with sick dogs
9	9	12	4	5

Table 5: Owner awareness on the zoonotic potential of leishmaniosis on the basis of the general information (period 2005 to 2010) (each number represents answer of one veterinary practice)

NEVER	SELDOM	PERIODICALLY	FREQUENTLY	CONSTANTLY
4	11	1	2	6

Table 6: Counsel frequency on zoonotic potential for the owners of dogs with leishmaniosis performed by veterinarians (period 2005 to 2010) (each number represents answer of one veterinary practice)

NEVER	SELDOM	PERIODICALLY	FREQUENTLY	CONSTANTLY
3	5	4	1	13

Zoonotic risk

Information on zoonotic risk are given in the tables 5 and 6

Majority of dog owners (62,5%) were rarely or never aware about the consequences for their health (Table 5). In contrast, 50 % of Slovenian veterinarians stated to constantly inform the owners of *Leishmania*-infected dogs on consequences for human health, while 46,2 % of them have given information periodically, seldom or never. None of veterinarians encountered a zoonotic case of canine leishmaniosis.

We can see that the majority of dog owners were rarely informed about the consequences for their health on the basis of information given by media during the concerned period.

Discussion

Due to the global climate changes and enhanced travel of dogs, borders of endemic areas with leishmaniosis in Europe have recently spread towards the north (8, 10). Higher environmental temperatures allow survival of sand flies belonging to the genus *Phlebotomus*, which are vectors for *L. infantum*. Likewise as the rest of the Europe also Slovenia has been able to observe changes of environmental temperature. Long-term (1961–1990) average summer temperature at Slovenian coast was 21°C. For the last 15 years regular drops below this average level have not been observed any more. That shows the average temperature since 1996 went higher. These data make serious concern about possibility of leishmaniosis spreading. Moreover Slovenia is located between two other Mediterranean countries (Italy and Croatia) where expansion of canine leishmaniosis is a concern.

The global data are based on the experience of 46,7 % of veterinary practices in the country, which makes a very high percentage. However the conclusions can be limited by the relatively low number of veterinary clinics in Slovenia that experienced the diagnosis of the disease.

Amongst the responders, 85,7 % haven't diagnosed a case of leishmaniosis in a dog during the period between 2005 and 2010. All responders shared opinion of not being practicing in enzootic area since they had never seen an autochthonous case. Half of veterinarians believed that number of CanL cases was even getting lower during that

period and 50% other believed that prevalence had remained unchanged. Results of this study can be compared to another, done in an enzootic area of southeastern Spain (surface 28000 km² and 5,5 million inhabitants while surface of Slovenia is 20273 km² with 2 million inhabitants). In this Spanish survey the percentage of responders was comparable to our survey (47 % and 46,7 %, respectively). Percentages of confirmed Spanish cases ranged from 13 % to 25 % of all dogs examined in veterinary practices during the one-year period, with only 2 % of veterinarians reporting no confirmed cases. Ninety one percent of Spanish responders believed that confirmed CanL cases had become infected within their working area and 58 % of veterinarians believed that local prevalence of leishmaniosis had increased over the preceding ten years and 25% believed that prevalence had remained unchanged (22). In contrast, Slovenian veterinarians observed, that the canine leishmaniosis case numbers are descending or at least stagnating.

It is important for veterinarians to recognize clinical symptoms of leishmaniosis. Most common are skin lesions that are reported in up to 60 % of cases (11, 23). Slovenian veterinarians have constantly to frequently observed skin lesions in CanL, such as alopecia, exfoliative dermatitis, ulcers, nodules, pyoderma, lesions on the bridge of the nose (depigmentation, ulcers) and lesions on footpads (excessive nail growth, hyperkeratosis, ulcers). They have frequently observed eye and eyelid lesions like periocular alopecia, nodules at the edge of the eyelid, conjunctivitis and uveitis. Beside this, non-dermatological signs such as apathy, elevated body temperature, anaemia, weight loss and diarrhea were frequently observed (Table 1). Apathy and weight loss are usually mentioned as consequences of anemia due to lower erythropoiesis associated to the chronic course of leishmaniosis itself or chronic renal failure with lowered erythropoietin production (23). Chronic renal failure is the most common complication of leishmaniosis and also the most common reason for death in dogs (12). Weight loss can be observed in about 25,3 % – 32 % dogs with leishmaniosis (24). Slovenian veterinarians have described weight loss as a constant, very frequent or frequent clinical sign in dogs with leishmaniosis. They have frequently observed signs of chronic renal failure. These results are in accordance to the literature data since histological changes of renal tissue had

been found in 100 % of dogs with leishmaniosis (14). One of the most common clinical sign in CanL is lymphadenopathy which was reported to be present in 65,2 % - 88,7 % of the cases (24). From table 1 it can be seen that Slovenian veterinarians have frequently observed lymph nodes enlargement in dogs with leishmaniosis. Periodically bone and joint changes were observed although leishmaniosis has been rarely described as a cause of lameness in dogs (23). Epistaxis was observed in more than 50 % of Slovenian practices that have diagnosed a CanL case from 2005 to 2010. The result is comparable to surveys in endemic area where 54 % of veterinarians frequently to very frequently observed epistaxis (22). Epistaxis has been described in the literature (5) as a rare but quite suggestive symptom that may be present in about 4 – 10 % of the cases (11).

Amongst serologic tests indirect immunofluorescence antibody test (IFAT) is described as the recommended golden standard method for the diagnosis of clinical cases (25). This method had been used only by one veterinary practice in Slovenia. ELISA test had been used more frequently and that is comparable to results of the study done in Spain (22). ELISA and IFAT are highly sensitive and specific tests for clinically expressed leishmaniosis but are not adapted for confirming asymptomatic dogs (26). Dogs that can express strong cellular immunity are able to control the reproduction of the parasite. Symptoms in these dogs consequently will not appear and they will express no to low antibody titer (low humoral immune response) (27). Slovenian veterinarians had used IDEXX SNAP[®] Canine Leishmania Antibody Test Kit which is simple for use and is reported to have a high specificity of 90,0-94,7 % and a high sensitivity of 94,7 % (28). Rapid tests are thus useful for diagnosis of sick dogs but do not detect dogs with low antibody levels or asymptomatic dogs. Moreover positive tests are only the first step, as they have to be followed by a quantitative serology for precise evaluation of prognosis and therapy.

Cytology of skin and lymph nodes had been periodically to seldom used by only two Slovenian veterinary practices. In dogs with clinical course of leishmaniosis low to moderate number of parasites can be found (29). Sensitivity of this method can be enhanced by selection of the tissue (the best is skin, popliteal lymph nodes, bone marrow and spleen) (16). Laboratory can enhance the sensitivity of cytological method by expertise or other

techniques like immunohistochemistry staining or immunofluorescence on biopsies, to achieve sensitivity from 70 – 100 % (27).

Polymerase chain reaction (PCR) is the most sensitive technique test to detect *Leishmania*. It uses detection of parasite DNA and may be used on different samples like conjunctival swabs, skin tissue, bone marrow aspirates, lymph node, spleen tissue or blood. Sensitivity of this test usually gets lower with the order of tissues listed above (30). Veterinarians of two Slovenian veterinary practices (28,6 % of all that had diagnosed CanL during the period from 2005 to 2010) have stated to use this method frequently to constantly and they have sent blood samples for PCR, although other tissue then blood would yield to higher sensitivity (30). A positive result by PCR means the presence of the parasite in the sample but not necessary a causative relation with the clinical suspicion and a negative PCR means the DNA was not present in the sample submitted. For these reasons PCR is only a second line diagnostic test after serology to detect the disease. Amongst the different techniques the only recommend are quantitative PCR on kinetoplast DNA (kDNA) (5).

In table 2 drugs that have been used by Slovenian veterinarians in the treatment of dogs with leishmaniosis in the period from 2005 to 2010 are listed. None of nowadays-available medicaments is able to completely eliminate the parasite from the body but can improve clinical state of the dog (31). Meglumine antimoniate (Glucantime[®]) and allopurinol are drugs of choice and they should be used in combination. Allopurinol is a "leishmanio-static" drug and when used alone the percentage of expected relapses after cessation of the treatment is high (32). The possibility to use Allopurinol alone in the treatment of Canine Leishmaniosis is limited to mild forms of the disease (5) which were not the majority of the stages of the disease diagnosed in Slovenia. Meglumin antimoniate is still used rarely in Slovenia because of its high price and inconvenient (parenteral) mode of use. On the contrary, allopurinol is a cheap drug used orally and because of that lots of veterinarians use it alone (12). That was the case also in Slovenian practices in the period from 2005 to 2010. Only one practice had periodically used meglumine antimoniate (Glucantime[®]). Effectiveness and safety of Miltefosine was recently reported but couldn't have much influence on the treatment protocols in the period from 2005 to 2010 (33).

One third of Slovenian veterinary practices had euthanized their cases in spite of the treatment during the period from 2005 to 2010. This percentage is not surprising taking into consideration that used treatment protocols were not optimal. Dog owners have decided for euthanasia most often because of chronic renal failure (CRF) and sometimes because they have refused the treatment (also because of the cost) and because of the presence of children in the household. Veterinarians have most often elected euthanasia because of CRF (table 3) and zoonotic potential. Recent report showed a lack of evidence that dog culling could diminish visceral leishmaniosis transmission. It was proposed, that animal culling as a control measure of human visceral leishmaniosis should be abandoned (34).

Chronic renal failure is in the literature described as the most common reason for euthanasia (12). In a study performed in France in the period 2002-2004 based on the information obtained from 547 veterinary clinics only 20% of dogs were finally euthanized (8).

High percentage of Slovenian practices that have suggested preventive use of insecticides and repellents (92.3 % of responders) in the period from 2005 to 2010 shows high awareness of Slovenian veterinarians in spite of the fact, that majority of them have never had a case of leishmaniosis. The results of this study are comparable to the study mentioned, that was done in endemic region of Spain, where 92 % of veterinarians suggested use of repellent collars and 74 % suggested spot-on preparations (22). The insecticides that were suggested by Slovenian veterinarians were amongst the most effective (7). Beside chemicals, staying in the house during the night was the most frequently suggested preventive measure by Slovenian veterinarians (18,4 %). Alternative measures (that were mostly experimental in the research period) were not suggested by Slovenian veterinarians opposite to Spanish ones, that have suggested these methods in as high as 25 % although 11 % of them doesn't believe in the efficacy of preventive measures at all (22).

In tables 5 and 6 it can be seen that dog owners were rarely informed on their health consequences from different public media. That can be understood since Slovenia is not known as an endemic region and media very rarely (if at all) publish anything on leishmaniosis. Opposite to general information, a quarter of Slovenian practices (50 % of

responders) have constantly informed dog owners on human health consequences.

Conclusions

The results of this survey, based on the high participation rate of veterinary clinics, show that most veterinarians in Slovenia have good background knowledge on the canine disease and inform the dog owners on the risk for exposed dogs (prevention) or for human beings. This survey also reveals the limits of the diagnostic methods as quantitative serology remains rarely used on suspected cases. Moreover treatment procedure remains frequently limited to the use of allopurinol for both economic and tolerance reasons resulting in a relatively elevated percentage of failure, relapse complications and finally euthanasia.

Most of Slovenian veterinary clinics did not encounter canine leishmaniosis in the period from 2005 to 2010. They all claimed that they do not work in an enzootic or endemic area, since that they did not come across any indigenous cases of this infection. This therefore shows that at least until 2010, Slovenia cannot be considered as an enzootic area. However an increase of travelling dogs to the other Mediterranean countries may result in an increase of the probability of the diagnosis on imported cases. Moreover in a context of very low endemicity the recognition of rare cases can be difficult and only serological or PCR based studies could precise the actual prevalence of the parasite in dogs in Slovenia. We propose for further epidemiological research of canine leishmaniosis in Slovenia, the utilization of diagnostic tests such as IFAT and PCR on a broad population of healthy and diseased dogs, together with vector analysis.

References

1. Desjeux P. Leishmaniasis: public health aspects and control. *Clin Dermatol* 1996; 14: 417-23.
2. Banuls AL, Hide M, Prugnolle F. Leishmania and the leishmaniasis: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. *Adv Parasitol* 2007; 64: 1-4.
3. Delgado O, Castes M, White AC, et al. *Leishmania colombiense* in Venezuela. *Am J Trop Med Hyg* 1993; 48: 145-7.
4. Dedet JP. Epidemiology of the European foci of human leishmaniosis. In: *Proceedings of the*

- 2nd CVBD World forum. Sicily, Italy 2007; 64–7.
5. Solano-Gallego L, Koutinas A, Miro G, et al. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. *Vet Parasitol* 2009; 165: 1–18.
6. González U, Pinart M, Reveiz L, Alvar J. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev* 2008; 4: 1–40.
7. Noli C, Auxilia ST. Treatment of canine Old World visceral leishmaniasis: a systematic review. *Vet Dermatol* 2005; 16: 213–32.
8. Bourdeau P, Mallet M, Marchand A. Canine leishmaniosis in France: treatment used and criterias of efficacy. In: *Proceedings World Association for the Advancement of Veterinary Parasitology*. Gent, 2007.
9. Maroli M, Rossi L, Baldelli R, et al. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Trop Med Int Health* 2008; 13: 256–64.
10. Bourdeau P. Canine leishmaniosis: the new situation. In: 23. Simpozij o aktualnih boleznih malih živali. Separati referatov: predkongresni dan - dermatologinja. Dolenjske Toplice, 2010: 6–8.
11. Ciaramella P, Oliva G, Luna RD, et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. *Vet Rec* 1997; 141: 539–43.
12. Roze M. Canine leishmaniasis : a spreading disease: diagnosis and treatment. *Eur J Companion Anim Pract* 2005; 15: 39–52.
13. Peña MT, Naranjo C, Klauss G, et al. Histopathological features of ocular leishmaniosis in the dog. *J Comp Pathol* 2008; 13: 32–9.
14. Zatelli A, Borgarelli M, Santilli R, et al. Glomerular lesions in dogs infected with *Leishmania* organisms. *Am J Vet Res* 2003; 64: 558–61.
15. Solano-Gallego L, Miró G, Koutinas A, et al. LeishVet guidelines for the practical management of canine leishmaniosis. *Parasites Vectors* 2011; 4: 86. <http://www.parasitesandvectors.com/content/4/1/86>
16. Maia C, Campino L. Methods for diagnosis of canine leishmaniasis and immune response to infection. *Vet Parasitol* 2008; 158: 274–87.
17. Saridomichelakis MN, Mylonakis ME, Leontide LS, et al. Evaluation of lymph node and bone marrow cytology in the diagnosis of canine leishmaniasis (*Leishmania infantum*) in symptomatic and asymptomatic dogs. *Am J Trop Med Hyg* 2005; 73: 82–6.
18. Ribeiro RR, Moura EP, Pimentel VM, et al. Reduced tissue parasitic load and infectivity to sand flies in dogs naturally infected by *Leishmania (Leishmania) chagasi* following treatment with a liposome formulation of meglumine antimoniate. *Antimicrob Agents Chemother* 2008; 52: 2564–72.
19. WHO. Advances in the battle against leishmaniasis. *TDR News* 1998; 57: 2.
20. Dantas-Torres F, Brandão-Filho SP. Visceral leishmaniasis in Brazil: revisiting paradigms of epidemiology and control. *Rev Inst Med Trop* 2006; 48: 151–6.
21. Mulić R, Custović A, Ropac D, et al. Occurrence of visceral and cutaneous leishmaniasis in Croatia. *Mil Med* 2009; 174: 206–11
22. de Ybáñez RR, del Río L, Martínez-Carrasco C, et al. Questionnaire survey on canine leishmaniosis in southeastern Spain. *Vet Parasitol* 2009; 164: 124–33
23. Bourdeau PJ. Update on canine leishmaniosis: from infection to optimized management. In: *New insights of infectious and parasitic dermatoses*. In: *Proceedings of the Bayer pre-congress symposium and 23rd European Congress of Veterinary Dermatology*. Bled, 2009: 10–27.
24. Baneth G. Leishmaniasis. In: Greene GE, ed. *Infectious diseases of the dog and cat*. 3rd ed. Philadelphia: W.B. Saunders, 2005: 685–95.
25. Ferrer L, Aisa MJ, Roura X, Portús M. Serological diagnosis and treatment of canine leishmaniasis. *Vet Rec* 1995; 136: 514–6.
26. Mettler M, Grimm F, Capelli G, Camp H, Deplazes P. Evaluation of enzyme-linked immunosorbent assays, an immunofluorescent antibody test, and two rapid tests (immunochromatographic-dipstick and gel tests) for serological diagnosis of symptomatic and asymptomatic *Leishmania* infections in dogs. *J Clin Microbiol* 2005; 43: 5515–9.
27. Saridomichelakis MN. Advances in the pathogenesis of canine leishmaniosis: epidemiologic and diagnostic implications. *Vet Dermatol* 2009; 20: 471–89.
28. Marcondes M, Biondo AW, Gomes AA, et al. Validation of a *Leishmania infantum* ELISA rapid test for serological diagnosis of *Leishmania chagasi* in dogs. *Vet Parasitol* 2011; 175: 15–9.
29. Moreira MA, Luvizotto MC, Garcia JF, Corbett CE, Laurenti MD. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different

clinical signs. *Vet Parasitol* 2007; 145: 245–52.

30. Reis AB, Martins-Filho OA, Teixeira-Carvalho A et al. Systemic and compartmentalized immune response in canine visceral leishmaniasis. *Vet Immunol Immunopathol* 2009; 128: 87–95.

31. Baneth G, Shaw SE. Chemotherapy of canine leishmaniasis. *Vet Parasitol* 2002; 106: 315–24.

32. Cavaliero T, Arnold P, Mathis A, et al. Clinical, serologic, and parasitologic follow-up after long-term allopurinol therapy of dogs naturally infected with *Leishmania infantum*. *J Vet Intern Med* 1999; 13: 330–4.

33. Miró G, Oliva G, Cruz I, et al. Multicentric, controlled clinical study to evaluate effectiveness and safety of miltefosine and allopurinol for canine leishmaniasis. *Vet Dermatol* 2009; 20(5/6): 397–404.

34. Costa CH. How effective is dog culling in controlling zoonotic visceral leishmaniasis? A critical evaluation of the science, politics and ethics behind this public health policy. *Rev Soc Bras Med Trop* 2011; 44(2): 232–42.

LEJŠMANIOZA PRI PSIH V SLOVENIJI, KI JO POVZROČA *LEISHMANIA INFANTUM*: ANALIZA PODATKOV, PRIDOBLENIH S POMOČJO ANKETIRANJA

T. Kotnik, K. Ahačič, A. Rostaher, P. Bourdeau

Povzetek: Lejšmanioza je bolezen, ki je posledica okužbe z različnimi vrstami protozojskega zajedavca iz rodu *Leishmania*. Od vseh je najbolj razširjena in raziskana okužba psov z vrsto *L. infantum*, ki je nalezljiva tudi za ljudi. Namen naše naloge je bil raziskati stanje lejšmanioze v Sloveniji za preteklo obdobje petih let, od leta 2005 do 2010. Ankete smo poslali 105 klinikam in dobili 49 odgovorov. Izmed tistih, ki so na anketo odgovorili, jih 42 (85,7 %) v zadnjih petih letih pri psih ni diagnosticiralo lejšmanioze. Večina slovenskih veterinarjev meni, da se tendenca novih primerov kot tudi tendenca kontrolnih pregledov psov, obolelih za lejšmaniozo, v obdobju petih let v Sloveniji ni poviševala, temveč je ostajala nespremenjena oziroma je celo upadala. Vsi psi z lejšmaniozo, ki so jih slovenski veterinarji odkrili v preiskovanem obdobju, so bili z že izraženimi kliničnimi znaki pripeljani iz držav z endemično obliko bolezni, večinoma iz Španije in Francije ter občasno iz Portugalske, Italije, Hrvaške in Afrike. Skoraj vedno so slovenski veterinarji opazili kožne spremembe, in sicer brezplačna mesta, ekfoliativni dermatitis, ulkuse na koži, kožne vozličke, gnojno vnetje kože, spremembe na nosu (depigmentacija, razjede) ter spremembe na blazinicah (hiperkeratoza, razjede). Pogosto so opazili tudi spremembe na očeh in vekah, kot so brezplačna mesta na vekah, vozlički na robu veke, konjunktivitis in uveitis, pa tudi apatičnost, povišano telesno temperaturo, slabokrvnost, hujšanje in drisko. Tretjino psov z lejšmaniozo so kljub zdravljenju usmrtili, največkrat zaradi odpovedi ledvic in zoonotskega potenciala. Rezultati naše raziskave so pokazali, da Slovenije ni mogoče prištevati med države z endemično obliko bolezni v obdobju do leta 2010. Povečana pogostnost potovanj v mediteranske države pa povečuje verjetnost obolevanja. Za prihodnje študije predlagamo uporabo vektorske analize skupaj z diagnostičnimi testi IFAT in PCR na večjem številu zdravih in obolelih psov.

Ključne besede: lejšmanioza; pes; *Leishmania infantum*; zoonoza; peščena muha