Chapter 1

Living on the Edge: Border Countries Should Have Strict Veterinary and Health Policy on Leishmaniasis

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Additional information is available at the end of the chapter

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Abstract

First human and canine cases as well as presence of competent Phlebotomine fly vectors are reported for the first time in Slovenia. Number of infected dogs in Slovenia has been increasing in the last few years. Having increased number of infected dogs and a presence of proven Phlebotomine fly vectors at the same time in a climatically suitable region may lead to endemic spread of the disease. And that is the kind of situation calling for governmental regulation. Basic preparedness and rapid response mechanisms should be in place. Leishmaniasis cases should be detected early and reaction should be quick. In epidemic-prone areas and before the anticipated outbreak season, the responsibilities of the outbreak task force members should be defined; the necessary needs for response, surveillance, and control should be assessed; the surveillance system should be reinforced; criteria for epidemic alert should be set up; and all health facilities should be provided with minimum stocks of basic diagnostic and treatment supplies. Successful preventive measures should include regular veterinary checks of all imported dogs and dogs traveling outside the country, vector control, use of effective repellents, sleeping indoors with nets on the windows, and antileishmanial vaccination of dogs.

Keywords: leishmaniasis, CanL, Phlebotomine, visceral leishmaniasis, *L. infantum*, *P. perniciosus*

1. Introduction

Leishmaniasis is a disease that affects humans, as well as wild and domestic animals. They are caused by parasites of the genus *Leishmania* (protozoa, trypanosomatidae) and are transmitted by Phlebotominae flies (Diptera, Psychodidae). Not all species of *Leishmania* parasites are of

medical importance; over 20 of them are considered pathogenic for humans and 10 have been isolated from dogs, including *L. infantum* (Syn. *L. chagasi*), *L. donovani*, *L. tropica*, *L. major*, *L. arabica*, *L. amazonensis*, *L. mexicana*, *L. braziliensis*, *L. peruviana*, and *L. colombiensis* [1]. *L. donovani* is the one that causes human visceral leishmaniasis (VL) with more than 90% of the cases occurring in India, Sudan, Bangladesh, and Brazil [2]. This species is involved in anthroponotic epidemiological cycle with anthropophilic vector and humans act as the reservoir of the disease. *L. tropica* causes anthropophilic cutaneous leishmaniasis (CL). Unique proven vector *Phlebotomus sergenti* is present mainly in urban areas, often at the periphery of old towns and cities, and in poor suburbs where low sanitary conditions facilitate breeding sites for this species. Zoophilic CL epidemics seem to be related to the fluctuations of the rodent populations and the accumulations of nonimmune people. This disease is found mainly in rural areas. The responsible parasite for zoonotic CL is *L. major* and the proven vector in the Mediterranean basin is *Phlebotomus papatasii* [3, 4]. *L. infantum* is involved in zoonotic epidemiological cycle with zoophilic vectors serving dogs as the reservoir of the disease [5] and is currently the predominant causative agent of VL in humans and dogs in the Mediterranean region. Majority of both suspected and proven vectors of this pathogen belongs to the *Larroussius* subgenus. With so many species of human-infective parasites, different reservoir and vector species in a wide range of topographically different foci, the ecology, and epidemiology of leishmaniases are without doubt the most diverse of all vector-borne diseases.

At the beginning of the twentieth century in Europe only sporadic human visceral leishmaniasis cases had been reported. The spread of the disease happened after 1975 and many of the European Union (EU) countries developed surveillance system around that time. The increased incidence of leishmaniasis in the Mediterranean region is due to several reasons including the influx of nonimmune population into the natural foci of transmission, changes in ecology of vectors and reservoir hosts, reduction in the use of residual insecticides for the control of mosquito populations, improvements in the diagnostic methods, and reporting of positive cases.

Human VL has long been considered a disease of young children but epidemiology of the disease after 1975 has changed with the increase of incidence in adults. This correlated to the emergence of HIV. However, in the last decade, numbers of VL infections in adults in many EU countries decreased. This can be attributed, among other measures, also to the use of a novel, highly active antiretroviral therapies (HAART) [6].

We are observing also changes of epidemiology of canine leishmaniasis (CanL). While foci of CanL including insecticide nontreated dogs of predisposed breeds traditionally were settled in the coastal districts, recent studies show that there are various risk factors for CanL, such as age 2 years or more, sleeping mostly outdoors, season of sampling (spring to autumn), and geographical origin [7, 8]. Today, leishmaniasis is endemic in all the countries of Southern Europe, with an incidence rate of 0.21 per 100,000 inhabitants and more than 750 autochthonous human cases reported each year [6, 9]. In the Mediterranean region, leishmaniasis is generally associated with *Leishmania infantum*, but other species autochthonous in Asia, the Middle East, and Africa, such as *L. donovani* and *L. tropica*, may colonize European Phlebotomine fly vectors as well.

Slovenia is one of the smallest member countries of the European Union neighboring to Austria, Italy, and Croatia. Despite an area count of only 16,423 square kilometers, it has diverse landscape. The northern part of the country is composed of Alpine and the southern part is composed of Mediterranean landscape. Being the bridge between eastern and western part of the Mediterranean, this region hosts unknown *Leishmania* species and some of the most important Phlebotomine fly vectors.

From 2004 to 2011 a questionnaire-based multinational survey on canine leishmaniasis has been conducted in Europe. Slovenian veterinary clinics were among the 12,546 subjects that have been questioned. Reply rate of Slovenian veterinarians was satisfying (46.7%) and the survey had shown no endemic CanL case recognized in Slovenia up to that time [10, 11]. In spite of its vicinity to Italy and Croatia, both well-known endemic countries, all infected dogs were found to be imported to Slovenia or have previously traveled to one of the endemic regions. Autochthonous cases of both canine and human leishmaniasis in Slovenia were not reported until recently. There may be two main reasons: unrecognizing and underreporting of potential cases.

In January 2014, the first endemic CanL case was reported in Slovenia the same year when the first specimens of Phlebotomine flies were collected. During the survey in the Istrian peninsula, both Slovenian and Croatian side, five Phlebotomine fly species were identified, including *P. neglectus* and *P. perniciosus*, some of the most important vectors of *Leishmania* parasites [12]. Although only one CanL autochthonous case and one suspected autochthonous human case (data not published) have been reported [13], recent information indicates increase in the number of infected dogs settled in Slovenia.

Registration of CanL cases in certain region is of great epidemiological importance for prevalence of disease in people as well.

2. Materials and methods

Data on CanL cases in Slovenia were collected via mail with a short and simple questionnaire that whether veterinarians had ever registered a CanL case that was born in Slovenia and that had never traveled outside the country. Mail was sent through the official mailing of Slovenian Veterinary Chamber in December 2015 and January 2016 to all veterinarians dealing with small animal practice.

Data on human cases in Slovenia were collected through the SURVIVAL program for official surveillance of communicable diseases supported by the Ministry of Health of Republic of Slovenia.

During 2013 and 2015, Phlebotomine flies were collected throughout the period of peak seasonal activity (15/05–31/09) at six sites from two diverse areas of Slovenia, including the coastline as well as the karst region (**Figure 1**).

Outdoors, Phlebotomine flies were collected by Centers for Disease Control (CDC) miniature light traps and sticky papers. CDC traps were operated all night, once per week in animal barns

and backyards and a cave. Two traps were set at each collecting site. The insect caught was checked each morning and the trapped flies were sorted and kept either dry or in 70% ethanol. Sticky traps were set in outbuildings, within walls, in tree holes in olive groves, and in the open surrounding phrygana-type scrubland. The trapped flies were removed with brushes and stored in 70% ethanol. Indoor collection of Phlebotomine flies was carried out using mouth and electric aspirators. The collected flies were mounted on permanent microscope slides for species identification, which was carried out according to keys by [14, 15].

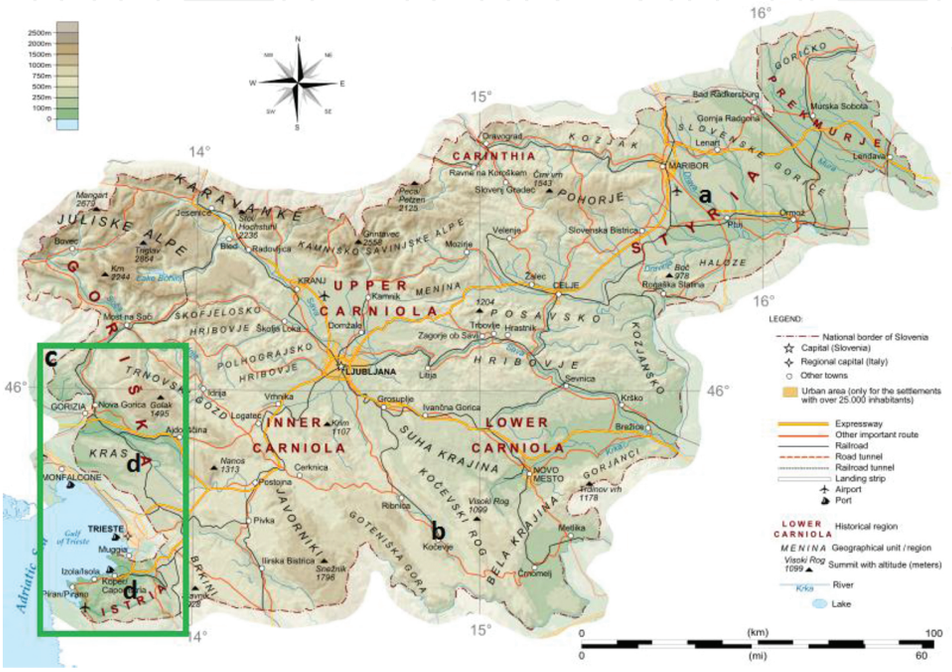

Figure 1. Map of the study area. **(a)** Veterinary clinic in Spodnji Duplek, **(b)** Kočevje, place of the first autochthonous case of canine leishmaniasis in Slovenia, **(c)** Slovenian littoral, and **(d)** Phlebotomine flies collection sites.

3. Results

Slovenian society, named "Hrtji svet" (Hounds world) has been rescuing dogs from Spain via adoption by Slovenian owners. During 2009–2016 they have imported 117 dogs. Although routine leishmaniasis testing on these dogs was suggested to all of the new owners, this could not have had prevented possible infective state of these dogs. In 7 of 117 dogs high antileishmania antibody titer was found and these dogs were treated with allopurinol. One dog died, and one has finished the therapy, but the rest five out of seven dogs were still under treatment at the time of writing this chapter. In Slovenia there is also another society that rescues dogs from Spain, but data on their numbers and epidemiological status are not available.

In **Table 1**, results on additional CanL cases, reported by one single veterinary practice, being settled in the North-East of the country are presented (**Figure 1a**). We can see that 14 seropositive dogs had been presented to the practice in the period 2012–2015. Nine of them had been tested because of clinical signs and five of them were found positive on routine testing.

No.	Gender/breed/age*	Clinical presentation**	IFAT	Electrophoresis
1	F/SG/8y	Routine testing	1:256	+
2	F/SG/3y	Skin changes	1:128	+
3	F/SG/6y	Skin changes, symmetrical lymphadenomegaly	1:256	+
4	F/SG/4y	Routine testing	1:256	+
5	F/SG/2y	Skin changes	1:256	+
6	M/SG/4y	Skin changes, symmetrical lymphadenomegaly gastrointestinal disturbances	1:128	+
7	M/SG/7y	Anorexia, epistaxis	1:128	+
8	M/SG/6y	Skin changes, symmetrical generalized lymphadenomegaly, pale mucous membranes, gastrointestinal disturbances	1:256	+
9	F/SG/5y	Routine testing	1:128	+
10	F/MIX/2y	Pale mucous membranes, skin changes , weight loss, lethargy	1:256	+
11	F/SG/3y	Anorexia, skin changes	1:128	+
12	F/SG/4y	Pale mucous membranes, skin changes	1:256	+
13	F/IH/3y	Routine testing	1:256	+
14	M/SG/4y	Routine testing	1:128	+

Abbreviations: F, female; M, male; SG, Spanish greyhound; IH, Ibizan hound; MIX, mixed breed.

Table 1. Seropositive dogs, adopted from Spain during 2012–2015, all successfully treated, settled mainly in Podravska (North-Eastern) region of Slovenia.

	P. neglectus		*P. perniciosus*		*P. papatasi*		*P. mascitii*		*S. minuta*		Total m/f	
2013–2015	m	f	m	f	m	f	m	f	m	f	m	f
	336	93	81	10	5	12	5	22	1	0	428	137
Total	429		91		17		27		1		565	

Abbreviations: m, male; f, female.

Table 2. Phlebotomine flies collected in the coastal and karst region of Slovenia (**Figure 1**) during 2013–2015.

One autochthonous CanL case was reported from South-Eastern region of Slovenia by a private practitioner. It was a female dog living in the South-East of the country (**Figure 1b**). This region has mild microclimate and is close to the Croatian border. The infected female dog regularly slept outdoors and had never left the country. The dog was presented to the local vet when it was 5 years old with signs of skin changes, loss of weight, general malaise, and changes in laboratory parameters, compatible to leishmaniasis (hypoalbuminemia, hypergammaglobulinemia, leukocytosis, monocytosis, and anemia). Immunofluorescence antibody titer (IFAT) test revealed 1:>160 antileishmania antibody titer. Clinical improvement was achieved following therapy with allopurinol.

Five human cases (two women and three men, 24–55 years old) were reported during the period from 1997 to 2016, two of them in 2015, and one of them in 2016.

During the period from 2013 to 2015, five species of Phlebotomine flies were collected and identified (**Table 2**).

4. Discussion

In this chapter, we present data on human leishmaniasis cases, CanL cases, as well as the presence of important leishmania Phlebotomine fly vectors in Slovenia. Among the five collected species that were found in the region, two of them *P. neglectus* and *P. perniciosus* are well known and proven vectors of *L. infantum* in the Mediterranean and two others *P. papatasii* and *P. mascitii* are known vectors of several phleboviruses [16]. Leishmania parasites were not isolated from Phlebotomine flies that were caught and tested in Slovenia. Nevertheless, since one autochthonous CanL case and at least one autochthonous human case were reported, we presume that there is a natural life-cycle of *Leishmania* sp. in the coastal region of Slovenia. Although other infection routes are proved in dogs (placental route, by sexual intercourse, by blood transfusion, and by infected flea bites) [17–20] they seem unlikely in our CanL case. The female dog was born in the region and had never traveled outside the country. It never got blood transfusion. It regularly slept outdoors. Clinical state of the mother and the spouse of the female dog (it got litter once), concerning leishmaniasis, was unknown but it is very likely that the female dog has been infected by Phlebotomine fly bite. The presence of infected vectors in Southeastern part of Slovenia is therefore highly probable.

Concerning the Mediterranean region, Phlebotomine fly's distribution pattern is quite bimodal. Western population was and it still is under direct influence of the African lineages. On the other hand, Eastern population is under the influence of both African and Asian continents. Northern part of the Mediterranean land mass is characterized by two narrow passages which directly influence spreading of Phlebotomine flies species. First one is restricted by Maritime Alps in the Southwestern part of the Alps, on the border between France and Italy. The second one and much more important for the Phlebotomine fly distribution is Slovenian Littoral, the westernmost part of Slovenia, bordering with the Italian region of Friuli-Venezia Giulia, true contact zone between Eastern and Western populations. It is about 13 km wide, karst plateau, a unique geological formation displaying distinctive surface features, containing cenotes, sinkholes, or dolines [21].

As a bridge between East and West, the region of Slovenian Littoral hosts unknown number of Phlebotomine fly species. Until now we have found some of the most important vectors of *Leishmania* parasites in the Mediterranean, including *Phlebotomus neglectus*, *P. perniciosus*, and *P. perfiliewi*, species that already have penetrated this narrow land bridge (**Figure 1**). Nevertheless, due to both environmental transformations and human activities there could be some other, more neglected ones such as *P. kandelaki* proven vector of *Leishmania* parasites in the Middle East.

Generally, most of the Phlebotomine fly species belonging to the subgenus Larroussius are potential vectors of *L. infantum*, and evidently due to rapid climatic changes, two of these are showing rapid aerial expansion. In 2003, the westernmost point of the range of *P. kandelaki* was in Montenegro [22], while in 2008, it was collected further to the west, at the coast of Croatia in Krk island (V. Ivović, unpublished data). Another fast spreading species and even more important vector, *P. neglectus* regularly situated in the Balkans, was recently found near Budapest (Hungary) [23]. Thanks to the attention recently drawn to the formerly neglected discipline of medical entomology, the latest results show the widening of the Phlebotomine fly distribution range to regions where they have never been found before.

Sixteen symptomatic dogs in Slovenia in the population of 220,700 officially registered dogs at the end of 2015 seem to be a small number. Nevertheless, point of view turns different taking into consideration that majority of the infected dogs never developed clinical symptoms. Incidence rate in endemic regions is usually less than 10% while seroprevalence can be as high as 90% [24]. We can speculate that the number of infected dogs in Slovenia is already higher in the moment. As many as 65% of asymptomatic dogs can harbor circulating parasites in their blood and as high as 93% of asymptomatic dogs are competent to transmit Leishmania to the vector, therefore these dogs allow transmission and spread of the disease [25, 26]. Beside dogs, other animals such as rats, cats, horses, rabbits, foxes, and jackals can be infected and may serve as a reservoir [12, 24, 27, 28]. Import of infected dogs to nonendemic regions creates one of the main risk factors for the spread of the disease. Many infected stray dogs have been brought to the north of Europe by compassionate tourists as well as social rescuing societies. There is an estimate that about 20,000 dogs infected with Leishmania presently live in Germany [29]. From the Crete and Cyprus example we can learn and predict increase of seropositive dogs in the Slovenian population. Seroepidemiological studies in dogs on island Crete, during the last 25 years, showed that the number of seropositive animals has been increasing [30]: from 0.27% in 1990 (data of the Greek Ministry of Agriculture) to 2.9% in 1994 and 19.8% in 2009. Same happened in Cyprus with a ninefold increase of seropositivity in dogs in the last 10 years [8]. This may be explained by the fact that dogs are brought into the island from mainland, especially from Attica, where leishmaniasis is endemic. The number of seropositive dogs in Crete continues to increase every year [30]. Another example is a situation that recently emerged in Australia. Before 2004, Antarctica and Australia were the only continents in the world that were free of leishmaniasis. In 2006, Biosecurity Australia ordered mandatory serological testing of dogs stationed in quarantine prior to importation. Few positive cases have been recognized until now, but there is concern of possibility of higher numbers [31]. Incubation time in leishmaniasis may be quite prolonged and clinical signs are not specific; therefore, veterinarians in nonendemic countries might overlook it.

Seroprevalence in people and dogs in Slovenia has not been estimated yet. High seroprevalence ratios in dogs were recently found in neighboring countries such as Italy and Croatian region of Dalmatia (21% and 42.85%, respectively) [32, 33]. Unfortunately, no information on the prevalence of healthy human inhabitants from Dalmatia is available. Recent data on seropositivity of human population residing in the Istrian region nearby the southern Slovenian border confirm the possibility of spreading the disease toward the north of Europe, including

Slovenia [34]. Moreover, seropositivity of Austrian inhabitants, living nearby the northern Slovenian border [35], indicates that the spread might have already happened. A potential vector *Phlebotomus mascitii* was found in this region [36]. Although *P. mascitii* is only an assumed vector of *Leishmania* spp.—data on its experimental transmission capacity are still lacking—the wide distribution of Phlebotomine flies in Austria, a country thought to be free of these insects, further supports a potential emergence of endemic leishmaniasis in Central Europe. Studies from France showed that *P. perniciosus*, species that was present in Slovenia too, is the most common leishmania vector in regions at low altitudes (less than 600 m above sea level). This suits to the spread of the disease in the Slovenian coastal region. Another species *P. ariasi* was found in France as the main vector at the attitudes between 200 and 1400 m above sea level [6]. If this species would spread to Slovenia, it looks that mountain region would perfectly suit it. Until now the presence of this species in Slovenia has not been proved.

Epidemiological studies show that incidence of human infections are directly related to the number of infective dogs and the presence of suitable vectors in the region. Control of reservoirs by dog culling is, apart from being expensive and time consuming, also not efficient. Because the breeding sites of Phlebotomine flies are unknown, control measures are focused mostly on adults [37]. There are several strategies targeting adult vectors. Nevertheless, applying environmental changes such as trimming trees and shrubs and cleaning and reorganizing in and around human dwellings and animal shelters can prevent favorable conditions for the development of Phlebotomine fly larvae. In some regions with high Phlebotomine flies control efforts have focused on the use of chemical insecticides, mostly on synthetic pyrethroids. Unfortunately, these measures, although initially attractive, are generally not permanent but are still most frequently used in controlling adult vectors. More advanced and sophisticated methods in Phlebotomine control includes planting of different plant species rich in phytochemicals that have a toxic effect against adult insects and larvae and use of bioinsecticides, particularly entomopathogenic against Phlebotomine flies [38].

Visceral leishmaniasis in people and dogs show similar clinical presentation involving intermittent pyrexia, lymphadenopathy, malaise, anemia, cachexia, hypergammaglobulinemia, and hepatosplenomegaly. Beside these dogs develop skin changes, mainly in the form of exfoliative and nodular dermatitis [39]. Hyperglobulinemia is thought to be related to a Th2-dominated immune response resulting in a marked humoral response and increased gamma globulin production [31].

Unfortunately, chemotherapy is not a successful measure in control of canine visceral leishmaniasis. Relapsing cases are common and drugs do not lead to the inhibition of infectivity to Phlebotomine flies [1]. Parasites were proved even from healthy looking skin of infected dogs. Unlike dogs, people cured of leishmania infections usually develop lifelong immunity. Treatment of VL in people is dependent on chemotherapy that should cure the patient and reduce the risk for relapse. The first-line drug for VL treatment should be liposomal amphotericin B or alternatively pentavalent antimonials and amphotericin B deoxycholate [40]. Miltefosine is being used on a compassionate basis in several European AIDS coinfected patients unresponsive to amphotericin B or pentavalent antimonials. Recently, this drug has been launched in the market for canine leishmaniasis treatment in Portugal, Spain, Italy,

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Greece, and Cyprus. Because dogs are never cured parasitologically and given the long half-life of the drug, the lack of European policy might contribute to the emergence of parasites resistant to miltefosine. Indeed, while this drug is successfully used in CanL treatment, there are reports on increasing incidences of relapse in humans on this treatment [41]. The same may happen with the use of ambisome in domestic pets that might produce spreading of resistant strains [3]. Like the Leish Vet group (international group of veterinary experts dealing with CanL), human experts conclude that chemotherapy alone would probably not be sufficient to eliminate the disease. Therefore, an effective vaccine should be developed and used in animals as well as in humans [40].

In the past, an effective vaccine against human leishmaniasis has already been used. This involved inoculation with live, virulent parasites in a process called leishmanization. It was practiced successfully in the former Soviet Union, the Middle East, and Israel. However, it was abandoned in most countries because of logistical problems and safety concerns, as some individuals developed nonhealing lesions and immune suppression [40, 42]. According to the authors' knowledge, no vaccine for routine use in people has been produced yet. The reason is that good understanding of immunity generated against pathogens is surely important for developing an effective vaccine. Current understanding of human immune responses generated against Leishmania parasites is mainly based on the studies in animal models and this cannot be simply extrapolated to humans [40]. While interferon-γ seems protective in mice and people, there have been enough of differences to prove that more studies in humans are needed. Patients developing visceral or diffuse cutaneous disease exhibit helper T-cell subtype 2 cytokine profile [13]. Genetically modified Leishmania parasites lacking essential genes such as dihydrofolate reductase, biopterin reductase, or cystine proteases have been shown to stimulate protection against challenge with virulent parasite strains in people. The main problem is the concern relating to safety and feasibility for large-scale use in the field [40]. Veterinary medicine probably gained an advantage in development of vaccines against leishmaniasis. A saponin formulation of fucose mannose ligand that is expressed throughout the lifecycle of a parasite was found to be safe, protective, and immunogenic and has become the Leishmune veterinary vaccine, licensed after a series of canine VL field studies [40]. In Europe, a vaccine based on the secreted-excreted antigen of *L. infantum* (CaniLeish, Virbac Animal Health) has been recently licensed, and has been available in some European countries since 2011. Some studies show good immunogenicity of this vaccine, although large-scale field studies are missing [43]. CanL vaccines proved to be efficacious not only in prevention of the disease but also in prophylactic manner, converting immune status of the infected dogs to more efficacious cell-mediated immunity, that is able to prevent visceral leishmaniasis [43]. We therefore agree with the authors Foroughi-Parvar and Hatam stating in their review article that the only efficacious method for control of CanL might be a vaccine [44].

The issue of notification of leishmaniasis differs in EU countries but even where notification is compulsory (i.e., Italy and Spain), notification of CanL cases is not a common practice. Generally, notification is compulsory in southern EU countries (Bulgaria, Greece, Italy, Portugal, and parts of Spain) but not in the part of EU, traditionally considered as nonendemic (France, Netherlands, etc.) [6, 45]. In Slovenia, notification of human case is mandatory in 3

days after the diagnosis [46] and record of CanL case should be reported to regional veterinary administration as well as to regional public health service as soon as confirmed by laboratory tests. These data are entered in the computer system of the Veterinary Administration of Republic of Slovenia monthly and reported twice annually to the World Organisation for Animal Health (OIE) via World Animal Health Information System (WAHIS). Notification of CanL cases in Slovenia is mandatory since 2014 but no CanL cases have been reported. Our data confirm that underreporting is taking place in Slovenia too, similarly to other border countries. Unrecognizing and underreporting of human and animal leishmaniasis in nonendemic countries can have wide-ranging consequences. Long reporting delay may happen even in endemic region like it was the case during recent community outbreak in Madrid (median of 151 days—41 days for visceral leishmaniasis and 183 days for cutaneous leishmaniasis). The delay arises from a number of factors that may be related to the patient (delay in seeking care) or the healthcare system (delay in diagnosis and reporting) [47].

Number of notified human cases in Slovenia at this moment is low. Interviews of these patients unfortunately were not done therefore no data were collected on clinical presentation nor traveling abroad. We even do not know whether reported cases were VL cases or cutaneous leishmaniasis cases. Nevertheless, having increased number of infected dogs and proven Phlebotomine fly vectors at the same time in climatically suitable region may lead to endemic spread of the disease [9]. That is a situation calling for governmental regulation. According to WHO's recommendations for epidemic-prone areas, basic preparedness and rapid response mechanisms should be in place. Leishmaniasis cases should be detected early and reaction should be quick. In epidemic-prone areas and before the anticipated outbreak season, the responsibilities of the outbreak task force members should be defined; the necessary needs for response, surveillance, and control should be assessed; the surveillance system should be reinforced; criteria for epidemic alert should be set up; and all health facilities should be provided with minimum stocks of basic diagnostic and treatment supplies [2].

Successful preventive measures in Slovenia and Slovenia-like border countries should include regular veterinary checks of all imported dogs and dogs traveling outside the country, vector control, combined to use of effective repellents, and sleeping indoors with nets on the windows, and importantly, antileishmanial vaccination of dogs.

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