

A Serologic Survey on Canine Leishmaniasis in Kocaeli, Sakarya, Mersin and Elazığ Provinces of Turkey

Utuk, A.E.,^{1*} Guven Gokmen, T.,² Bolacali, M.,³ Balkaya, I.⁴ and Simsek, S.⁵

¹ University of Cukurova, Faculty of Ceyhan Veterinary Medicine, Department of Parasitology, Adana, Turkey;

² University of Cukurova, Faculty of Ceyhan Veterinary Medicine, Department of Microbiology, Adana, Turkey;

³ University of Siirt, Faculty of Veterinary Medicine, Department of Animal Science, Siirt, Turkey;

⁴ University of Atatürk, Faculty of Veterinary Medicine, Department of Parasitology, Erzurum, Turkey;

⁵ University of Firat, Faculty of Veterinary Medicine, Department of Parasitology, Elazığ, Turkey.

* **Corresponding author:** Assoc. Professor Armağan Erdem UTUK, PhD., University of Çukurova, Faculty of Ceyhan Veterinary Medicine, Department of Parasitology, 01930, Adana, Turkey; Telephone: +90-322-6133507; Email: erdemutuk@hotmail.com

ABSTRACT

The aims of this pilot study were to determine the seroprevalence of canine leishmaniasis in Kocaeli, Sakarya, Mersin and Elazığ provinces and make a general evaluation of the serologic prevalence of Canine Leishmaniasis (CanL) in Turkey. For this purpose, 111 sera were collected from dogs of different breeds, ages and sexes. Anti-*Leishmania* IgG antibodies were detected with a commercially available IFAT kit. The relationship between location, breed, age, sex and the seropositivity were evaluated with Chi Square (X^2) test. At the end of the study, the overall seroprevalence was determined as 7.20% (8/111). Seroprevalance rates were 5%, 10.52% and 18.75% in Sakarya, Kocaeli and Mersin provinces, respectively; however, no antibodies were detected in dogs from Elazığ. There was no statistical difference in breed, sex and age groups ($P>0.05$). The prevalence of infection in the province of Mersin was significantly higher than in Elazığ ($p<0.05$). The mean seroprevalence of CanL was calculated as 7.29% for Turkey, which is similar to our results (7.20%). In this study, we gave information about the serostatus of CanL in four different provinces of Turkey. We consider that further studies are required for a clearer understanding of the parasite epidemiology and effective control measures.

Key words: *Leishmania*; IFAT; Dog; Seroprevalance; Turkey.

INTRODUCTION

Leishmaniasis is a vector borne protozoan disease that affects a wide range of mammalian host. Main vectors of *Leishmania* species are female sand flies of the genus of *Phlebotomus* and *Lutzomyia* (1). Disease is present in 98 countries of the world and important for human and animal health due to its zoonotic potential. The disease has three forms: visceral, cutaneous and mucocutaneous. Approximately 310 million people are at risk of the disease. 300,000 visceral and 1 million cutaneous leishmaniasis cases occur annually, and 20,000 to 40,000 of them die from the visceral form of the disease (2).

Leishmania infantum is the etiologic agent of canine leishmaniasis (CanL) in Turkey and endemic in the Aegean and Mediterranean Regions of the country (3). Lymphadenomegaly, weight loss, changes in appetite, lethargy, anemia, splenomegaly, polyuria, polydipsia, fever, vomiting, diarrhea, dermatitis, alopecia, onychogryphosis, blepharitis, conjunctivitis, keratoconjunctivitis, anterior uveitis, endophthalmitis, epistaxis, neurological and vascular disorders and lameness are the clinical signs of CanL (4). Disease is diagnosed by parasitological (cytology, culture, immunohistochemistry) serological (ELISA, IFAT, DAT,

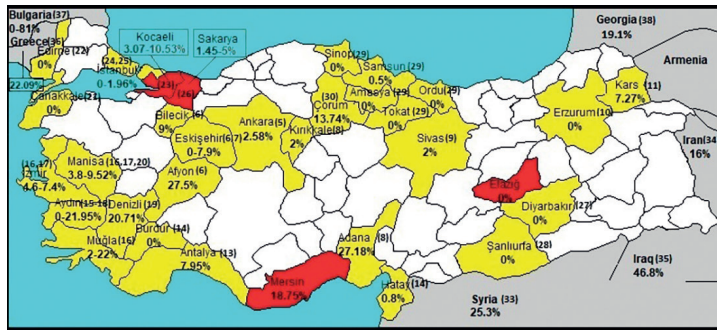


Figure 1: Seroprevalance rates of CanL in different parts of Turkey and neighboring countries. Areas shaded in red were covered in this study. Yellow and grey shaded areas were subject of previous studies.

rK39 based rapid test kits) and molecular (conventional PCR, Nested PCR, Real time PCR) tests (3,4).

A key point of an effective control program is to determine the serostatus of the disease both in animals and in humans. The goal of this study was to determine the seroprevalence of CanL in four different provinces of Turkey.

MATERIAL AND METHODS

A total of 111 blood samples were taken from the cephalic vein into sterile serum tubes from dogs in Kocaeli, Sakarya, Mersin and Elazığ provinces of Turkey (Figure 1). Bloods were collected from animal shelters (Mersin), owned dogs (Kocaeli and Sakarya) and free-roaming (Elazığ) dogs. Collected samples were left for clotting about 3 hours at room temperature, and then, centrifuged at 5000 rpm for five

Table 1: Seropositivity rates of Leishmania sp. with regard to age, gender, breed and location.

Variable	Category	No. of tested	No. of negative	No. of positive	% positive	Chi-Square Value	P-Value
Age	< 3	47	45	2	4.26	1.062	0.463
	≥ 3	64	58	6	9.38		
Gender	Female	76	71	5	6.58	0.142	0.705
	Male	35	32	3	8.57		
Breed	Pure	25	24	1	4.00	0.496	0.681
	Cross	86	79	7	8.14		
Location	Kocaeli	38	34	4	10.53 ^{ab}	6.833	0.042*
	Elazığ	37	37	0	0.00 ^b		
	Sakarya	20	19	1	5.00 ^{ab}		
	Mersin	16	13	3	18.75 ^a		
Total		111	103	8	7.20		

*: P<0.05.

a, b: Subscripts with different letters differ within same column significantly (P<0.05).

minutes. Obtained sera were stored at -20 °C until used. All dogs were clinically healthy and grouped according to their sex, gender, breed and location (Table 1).

A commercially available *Leishmania* IFAT Kit (Fuller Laboratories, California, USA) was used for the detection of anti-*Leishmania* antibodies. 1/40 and over sera titers were accepted as positive. The test was carried out according to the manufacturers recommendations.

The relation between location, breed, age, sex and the seropositivity was evaluated with Chi Square (X²) test. In the tables presented the data points bearing different letters are significantly different at P<0.05.

To determine the average seroprevalence of CanL in Turkey, serologic studies about disease were investigated. The numbers of animals tested and the number of seropositive animals were added, and percentage prevalences were calculated in respect of regions and the country (Table 2).

This study was approved by the Ethics Committee of Faculty of Ceyhan Veterinary Medicine (20.12.2017/E.53417).

RESULTS

At the end of the study, the overall seroprevalence was determined as 7.20 % (8/111). Seroprevalance rates were 5%, 10.52% and 18.75% in Sakarya, Kocaeli and Mersin provinces, respectively; however, no antibodies were detected in dogs from dog in Elazığ. There was no statistical difference in different breed, sex and age groups (P>0.05), but the seroprevalence rate between Mersin and Elazığ provinces was significantly different. (P<0.05) (Table 1).

The average seropositivities were 1.82% (8/439) for Central Anatolia, 5.06% (12/237) for Eastern Anatolia, 12.79% (71/555) for the Mediterranean region, 10.78% (163/1512) for the Aegean region, 11.83% (11/598) for Marmara, 0% (0/180) for Southeastern Anatolia and 5.12% (19/371) for Black Sea regions of Turkey. The overall seroprevalence was determined as 7.29% for Turkey (Table 2).

Table 2: Serological studies about Canine Leishmaniasis in Turkey.

Region/Mean seroprevalence	Province	Test	Cutt-off degree	No. tested	No. positive	% positive	Reference	
Central Anatolia 1.82% (8/ 439)	Ankara	IFAT	$\geq 1/128$	116	3	2.58	5	
	Eskişehir	IFAT	$\geq 1/64$	185	0	0	6	
	Eskişehir	IFAT, ELISA	$\geq 1/128$ for IFAT, \geq for ELISA	38	3	7.9	7	
	Kırıkkale	IFAT	$\geq 1/128$	50	1	2	8	
	Sivas	IFAT	$\geq 1/128$	50	1	2	9	
Eastern Anatolia 5.06% (12/237)	Erzurum	IFAT	$\geq 1/128$	72	0	0	10	
	Kars	IFAT	$\geq 1/128$	165	12	7.27	11	
Mediterranean 12.79% (71/555)	Adana	IFAT	$\geq 1/80$	206	56	27.18	12	
	Antalya	IFAT	$\geq 1/128$	176	14	7.95	13	
Aegean 10.78% (163/1512)	Burdur	IFAT	$\geq 1/128$	49	0	0	14	
	Hatay	IFAT	$\geq 1/128$	124	1	0.8	14	
	Afyon	IFAT, ELISA	$\geq 1/128$ \geq for ELISA	29	8	27.5	6	
	Aydın	IFAT, ELISA	$\geq 1/128$ for IFAT \geq for ELISA	109	10	9.1	15	
	Aydın	IFAT	$\geq 1/128$	31	0	0	16	
	Aydın	IFAT	$\geq 1/64$	41	9	21.95	17	
	Aydın	IFAT	$\geq 1/128$	78	11	14.1	16	
	Aydın	IFAT	$\geq 1/128$	253	42	16.6	18	
	Denizli	IFAT	$\geq 1/128$	140	29	20.71	19	
	İzmir	IFAT	$\geq 1/64$	108	8	7.4	17	
	İzmir	IFAT	$\geq 1/128$	65	3	4.6	16	
	Manisa	IFAT	$\geq 1/128$	26	1	3.8	16	
	Manisa	IFAT	$\geq 1/64$	42	4	9.52	17	
	Manisa	IFAT	$\geq 1/128$ for IFAT DAT ≥ 320 for DAT	490	26	5.3	20	
	Marmara 1.83% (11/598)	Muğla	IFAT	$\geq 1/128$	50	1	2	16
Muğla		IFAT	$\geq 1/128$	50	11	22	16	
Bilecik		IFAT, ELISA	$\geq 1/128$ \geq for ELISA	44	4	9	6	
Çanakkale		IFAT	$\geq 1/128$	27	0	0	21	
Edirne		IFAT	$\geq 1/128$	37	0	0	22	
Kocaeli		IFAT ELISA	$\geq 1/128$ for IFAT, \geq for ELISA	65	2	3.07	23	
İstanbul		IFAT	$\geq 1/128$	204	4	1.96	24	
Southeastern Anatolia 0% (0/180)	İstanbul	IFAT	$\geq 1/128$	152	0	0	25	
	Sakarya	IFAT	$\geq 1/128$	69	1	1.45	26	
	Diyarbakır	IFAT	$\geq 1/128$	100	0	0	27	
	Şanlıurfa	IFAT	$\geq 1/128$	80	0	0	28	
	Amasya	ELISA	20%	10	0	0	29	
	Çorum	IFAT	$\geq 1/128$	131	18	13.74	30	
	Black Sea 5.12% (19/371)	Ordu	ELISA	20%	10	0	0	29
		Samsun	ELISA	20%	200	1	0.5	29
Sinop		ELISA	20%	10	0	0	29	
Tokat		ELISA	20%	10	0	0	29	
Turkey				3892	284	7.29		

DISCUSSION

Leishmania species are found all over the world, except the Antarctica continent. Africa, Asia, Middle East, Latin America and the Mediterranean basins are endemic territories (31). Turkey is a bridge between Asia and Europe. Mediterranean, Black Sea and continental climates are observed in different parts of the country (32).

Leishmaniasis is endemic in the Aegean and Mediterranean Regions of the Turkey, but can be observed in any parts of the country as sporadic cases. Dogs suffer from CanL and are important reservoirs for sand flies and humans (3).

When comparing the data from this study to other studies, the average seropositivities of CanL ranges between 2% and 40% in the Mediterranean countries, including Turkey (13). In this study, the overall seroprevalence was determined as 7.20%. No anti-*Leishmania* antibodies were determined in Elazığ province (0%). The highest seroprevalance was observed in Mersin province (18.75%), and seroprevalence rates were 5% and 10.53 in Sakarya and Kocaeli provinces, respectively. The overall seroprevalence of our study is approximately the same as the average for Turkey (7.29%). However, when we make an individual comparison, it appears that Mersin is an endemic province with an average of 18.75%.

According to Özkan *et al.* (26), and Tamer *et al.* (23), the seroprevalances of CanL is 1.45% in Sakarya and 3.07% in Kocaeli provinces. In this study, the seroprevalence rates were higher for Adapazarı and İzmit (Table 1, 2). We could not detect anti-*Leishmania* antibodies in Elazığ province, which is in concordance with the results of the studies of the east and south-east provinces (Şanlıurfa, Diyarbakır and Erzurum) of Turkey with one exception, Kars (10, 11, 27, 28).

When we focus on the Turkish map (Figure 1), we can see that seroprevalance rates are higher in neighboring countries. For instance, the seroprevalences of CanL are 25.3% in Syria (33), in 16% Iran (34), 46.9% in Iraq (35), 22.09% in Greece (36) and 0-81% in Bulgaria (37); but, 0% in Şanlıurfa (28), Diyarbakır (27), Çanakkale (21) and Edirne (22) province of Turkey. We consider that further serosurveys should be carried out in places where the seroprevalence is 0%.

The differences in seroprevalence rates may be associated with different serologic tests, cut-off values, specificity and sensitivity rates and sampling (38, 39). Low cut-off values result in high sensitivity and low specificity while high values have the opposite effect (40). When we look at the Table 2,

we can see that same researchers use different cut-off values for the same tests in different studies. It should be kept in mind that non-optimized serological tests may result in false positivity/negativity, and erroneous sampling may have adverse effects on the test results.

With this study, we provide information on the serostatus of CanL in four different provinces of Turkey, and evaluate the seroprevalence rates at regional and country level. In conclusion, for the clear understanding of the parasites epidemiology and in order develop effective control strategies: i) reference laboratories and standard methods with the same cut-off values should be established, ii) studies should be carried on wild carnivores and rodents in addition to dogs, cats and vectors, iii) the records of disease determinants (age, breed, sex, climatic factors) should be well documented and v) further large scaled studies should be carried out in provinces which have not been examined before.

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