

Comparisons of Clinical Findings and Serological Data in the Diagnosis of Canine Leishmaniosis

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Abstract: This study aimed to evaluate clinical signs related with canine leishmaniosis (CanL) and to determine their relation with serological results in the differential diagnosis of this disease in dog populations.

A total of 253 dogs from houses and a dog shelter in Kuşadası, an endemic region for leishmaniosis, were investigated for clinical signs related to CanL. Sera collected from the study group were examined by indirect fluorescence antibody (IFA), whole ELISA and rK39 ELISA tests for the serological diagnosis. Popliteal lymph node aspiration materials were examined for the parasitological diagnosis.

Clinical signs were separated into two groups as cutaneous and visceral signs and no clinical signs were observed in 51.7% (131/253) of the dogs, while 48.3% (122/253) of the dogs had at least one sign. A total of 42 dogs [29 (23.7%) in the symptomatic and 13 (9.9%) in the asymptomatic group] were diagnosed as CanL serologically and/or parasitologically. Two strains were isolated and identified as *Leishmania infantum* MON-1. There was no correlation between IFAT titers and the number of the clinical signs ($P > 0.05$; R square = 0.002). Weight loss was found to be the most common sign of CanL while epistaxis was the least common but specific sign.

The seropositivity ratio of CanL is 16.6% (42/253) among dogs in the region and clinical signs were found to be helpful for suspecting CanL but at least one serological and/or parasitological method should be performed for the accurate and differential diagnosis in the dogs. Our findings also showed that the one-third of the dogs in an endemic area for CanL could be asymptomatic.

Key Words: Canine leishmaniosis, clinical signs, diagnosis, Turkey

Köpek Leishmaniosis Tanısında Klinik ve Serolojik Bulguların Karşılaştırılması

Özet: Bu çalışmamızda köpek leishmaniosis ile ilişkili olan klinik belirtilerin değerlendirilmesi ve köpeklerdeki bu hastalığın ayırıcı tanısında serolojik yöntemlerden alınan sonuçlarla ilişkisinin belirlenmesi amaçlanmıştır. Köpek leishmaniosis açısından endemik bir bölge olan Kuşadası'nda evlerde ve köpek evinde bulunan çeşitli ırklardan toplam 253 köpek, köpek leishmaniosisinin klinik belirtileri açısından incelenmiştir. İndirekt Floresan Antikor Testi (IFAT), düz ELISA ve rK39-ELISA serolojik testler olarak uygulanmış ve parazitolojik inceleme için popliteal lenf aspirasyonu yapılmıştır.

Klinik belirtiler, visseral ve deri belirtileri olarak iki gruba ayrılmış ve köpeklerin % 48,3'ünde (122/253) en az bir belirti saptanırken % 51,7'sinde (131/253) hiçbir belirti olmadığı görülmüştür. Toplam 42 köpeğe [septomatik köpeklerin 29 (% 23,7)'una ve asemptomatik köpeklerin 13 (% 9,9)'üne] serolojik ve/veya parazitolojik olarak köpek leishmaniosisini tanı konulmuştur. Aspirasyon örneklerinden iki suş izole edilmiş ve *Leishmania infantum* MON-1 olarak tanımlanmıştır. IFA testinde pozitiflik saptanan sulandırımın yüksekliği ile klinik belirtilerin sayısı arasında herhangi bir uygunluk saptanmamıştır ($P > 0,05$; R kare = 0,002). Kilo kaybı en yaygın, burun kanaması ise en az görülen ama özgül olan semptom olarak bulunmuştur.

Köpek leishmaniosisinin ülkemizin batısında yaygın (% 16,6) ve klinik belirtilerin bu hastalıktan şüphelenilmesinde yardımcı olduğu ancak kesin ve ayırıcı tanı için mutlaka serolojik veya parazitolojik testlere gereksinim duyulduğu görülmüştür. Ayrıca, köpeklerin 1/3'ünün de seropozitif olduğu halde asemptomatik olabileceğine dikkat çekilmiştir.

Anahtar Sözcükler: Köpek leishmaniosisi, klinik bulgular, tanı, Türkiye

Introduction

Visceral leishmaniosis (VL) caused by *Leishmania infantum* is a human zoonotic infection throughout the Mediterranean basin including Turkey. Dogs and rodents are accepted as the most important reservoirs, and the disease is transmitted by vector Phlebotomine sand flies (1-3). Canine leishmaniosis (CanL) is a common disease among dog populations in the Mediterranean area (3,4). Diagnosis of leishmaniosis in humans and dogs is crucial for controlling the disease and understanding its epidemiology. Direct microscopic examination and in vitro culture of tissue aspirates are still considered as the "gold standard" in the diagnosis, although it is a time consuming and invasive method (3). In the last decade, several serological tests with comparable rates of reliability have been developed and widely used especially in epidemiological surveys (5-8).

The clinical features of CanL vary widely. Skin lesions, loss of weight or poor appetite, local or generalized lymphadenopathy, ocular lesions, splenomegaly, fever, dermatitis, epistaxis, melena are commonly identified on physical examination of dogs. Cutaneous lesions characterized by hyperkeratosis, scaling, thickening, mucocutaneous ulcers and intradermal nodules on muzzle, pinnae, ears and footpads are detected in approximately 90% of the infected dogs (3,9).

CanL is very important as a fatal and non-curable disease of canines. Also, the persistence of the disease puts both healthy dogs and humans at risk in endemic sites. There have only been limited studies on CanL throughout Turkey. Seroprevalence of CanL was previously found to be between 3.6-19% in the villages of the Manisa city. Specific symptoms of CanL, such as enlargement of popliteal lymph nodes, skin lesions, depilation, weight loss and/or spontaneous death were observed in a limited number of seropositive dogs (8). In a study carried out in Kuşadası, 109 dogs kept at the dog shelter belonging to the Kuşadası Municipality were screened serologically using indirect fluorescence antibody test (IFAT) and recombinant K39-ELISA and the seropositivity ratio was found to be as 9.1% (10).

Although clinical features may be indicative and helpful for the diagnosis of the disease in dogs, due to the natural genetic heterogeneity of the canine population, these signs mimic those of other diseases (1,4). Serological diagnosis is considered to be essential for evaluating the prevalence of *Leishmania* infections especially among asymptomatic dogs (3).

This study aimed to evaluate the clinical signs related with CanL and to determine their relation with the serological results in the differential diagnosis of the disease.

Materials and Methods

Kuşadası was chosen as study area because of it is an important endemic region for canine leishmaniosis and has an importance as a tourist town of Turkey.

Clinical signs were evaluated in 253 dogs from Kuşadası / Aydın between the dates 1997 and 2001 and separated into two groups as visceral (fever, weight loss, fatigue, lymphadenopathy, conjunctivitis, epistaxis) and cutaneous (depilation, scaling, skin ulcer, onychogryphosis) signs (11).

The dogs showing these signs came from two different sources; (i) the dogs (Kangal, boxer, German shepherd, Doberman, terrier, Dalmatian, mix, etc.) serving as pets and guards in the residential houses and farms in urban area of Kuşadası and (ii) dogs living in the dog shelter belonging to Kuşadası municipality, located in the town. The dogs kept in the shelter were mix bred and came from urban and rural areas of Kuşadası.

Five ml venous blood samples were collected from each dog by brachial vein puncture for serological tests.

Serological Tests:

The antigen was prepared using promastigotes from local *Leishmania infantum* MON-1 strain obtained by mass culturing in RPMI-1640 containing 10% FCS.

Indirect fluorescence antibody test (IFAT) was performed using standard procedures and two-fold serial dilution from 1:16 to 1:16.384 of dog sera in PBS was used. Titers of $\geq 1:128$ were considered as positive for CanL and the titer of 1:64 was accepted as the borderline titer according to preliminary studies (6).

ELISA; was performed as described before, using 1:100 single serum dilution, rabbit anti-dog IgG conjugated with horseradish peroxidase (Sigma) at 1:1000 dilution and a 2, 2'-azino-bis (3-ethylbenz-thiozoline-6-sulfonic acid) (ABTS) substrate. The optical density was measured at 405 nm. Cut-off value was determined as the mean of the absorbance of two negative controls plus three standard deviations (12).

Recombinant K39 Enzyme Linked Immunosorbent Assay (rK39-ELISA); was performed as explained in

whole ELISA, except the purified rK39 antigen at a concentration of 20 ng per well (8).

Parasitological examination

A total of 66 popliteal lymph node aspirations were applied using 21G needles and examined under the microscope (x1000) after staining with Giemsa, and inoculated into NNN culture for isolating the parasite. Parasitological examination was accepted as positive with smear and/or culture positivity.

Statistical analysis

The correlation between IFAT titers and the number of the clinical signs were evaluated by regression analysis in SPSS for Windows.

Results

One or more visceral and/or cutaneous clinical signs were recorded in 48.3% (122/253) while no sign was observed in 51.7% (131/253) of the dogs.

A total of 42 dogs out of 253 were diagnosed as CanL serologically and/or parasitologically. Ninety-seven percent concordance between three tests was found and there was no correlation between IFAT titers and the number of the clinical signs ($P > 0.05$; R square = 0.002). The comparative results of the dogs according to clinical signs and the serological results are summarized in Table 1.

The 29 dogs confirmed with CanL were also grouped according to signs as follows: two dogs (6.9%) showed only one or two signs, 20 dogs (69%) showed three to five signs and seven dogs (24.1%) showed six to nine signs.

Popliteal lymph node aspirations were applied to 66 (34 from seropositive, 26 from seronegative and 6 from

borderline) dogs and 32 (31 from seropositive and 1 from borderline) aspiration samples were found to be positive with smear and/or culture examinations. Two strains were isolated and identified as *Leishmania infantum* MON-1 by isoenzyme analysis in Montpellier, France.

The percentage of clinical signs among 42 seropositive dogs was given in Table 2. Weight loss was found to be the most common sign of CanL while epistaxis was the least common sign. Conjunctivitis, epistaxis and onychogryphosis were found to be most specific signs of CanL.

Table 2. Clinical signs percentages among 42 seropositive dogs.

Clinical signs		n	%
Cutaneous signs	Skin ulcer	16	38
	Onychogryphosis	15	35.7
	Depilation	15	35.7
	Scaling	13	30.9
Visceral signs	Weight loss	21	50
	Fever	16	38
	LAP	14	33.3
	Fatigue	13	30.9
	Conjunctivitis	10	23.8
	Epistaxis	5	11.9
No sign		13	30.9

Discussion

The results obtained from the present work on the clinical signs of CanL gathered new data for understanding of the epidemiology of this infection in Turkey. In the present study, 30.9% (13/42) of the seropositive dogs showed none of the signs of CanL. This result indicates that epidemiological studies that only take symptomatic dogs into account should under-estimate the

Table 1. The relationships between group of signs and serological results in 253 clinically investigated dogs.

Clinical Signs	Serological Results			Total
	Seronegative n (%)	Borderline n (%)	Seropositive n (%)	
Visceral sign(s)	69 (94.5)	1 (1.3)	3 (4.1)	73
Cutaneous sign(s)	11 (91.7)	0	1 (8.3)	12
Visceral and cutaneous signs	11 (29.7)	1 (2.7)	25 (67.6)	37
No sign	109 (83.2)	9 (6.8)	13 (9.9)	131
Total	200	11	42	253

prevalence of infection in dogs by about one-third. This is in agreement with other studies, which concluded that a high proportion of infected dogs are asymptomatic. Therefore, serodiagnosis is absolutely essential in evaluating the prevalence of CanL in large dog populations (1,13,14) and in the diagnosis of individual cases (15).

Three commonly used serological tests were performed in the study because of reliable comparison with clinical signs and all three tests showed a 97% concordance and discriminated the positives equally. In previous studies performed in human VL endemic sites in Turkey, the CanL seropositivity rate was ranged from 3.6 to 19% in randomly selected dog populations (8). CanL seropositivity rate was found to be 9.1% among randomly selected dogs in the dog shelter of Kuşadası (10). However, the CanL rate for Kuşadası in this study was 23.7% (29/122) among dogs having clinical signs. Therefore, dogs with clinical signs found to have a positivity rate that is almost three times greater than a randomly selected population. These results indicated that the dogs having one or more of these clinical signs should be suspected for CanL as reported in a case (15) in Turkey. For an accurate diagnosis, serological examinations are more valuable, rapid and less invasive than parasitological procedures. Although epistaxis and conjunctivitis were found to be rare signs, they were found to be more specific than other common signs of CanL cases.

In the present study, 69% (29/42) of the seropositive group showed signs of CanL. In other studies, skin involvement was reported to be between 81 to 100% (4,16), while it was found to be 61.9% (26/42) and visceral involvement was seen in 67% (28/42) of the seropositive dogs in our study. One and 3 dogs were showed only cutaneous and visceral signs respectively while the majority group of seropositive dogs were showed visceral and cutaneous signs together (59.5%, 25/42) similar to an earlier report (11).

There was no correlation between the IFAT titers and the number of clinical signs ($P > 0.05$; R square =

0.002). This is also in concordance with earlier reports (2,6) and one of most valuable finding in the study in order to show importance of serological methods. The percentage of clinical signs of the dogs included our study are in concordance with the previously related literature (4,16).

Popliteal lymph node aspirations were done on 66 among 253 dogs to verify the reliability of the serological tests. None of the seronegative dogs and 31 out of 34 (91.1%) seropositive dogs were found to be positive by parasitological examination. Failure to detect parasites by parasitological examination in three out of 34 seropositive dogs and also in five out of six borderline dogs may be due to either very low parasite levels or the dog may have gone through a subclinical infection in the past. There is a need to control borderline dogs with regular intervals.

In the present study, euthanasia was performed to 19 out of 42 seropositive dogs by an authorized veterinary surgeon following the permission of their owners while nine of them died spontaneously. Drug therapy with antimonials is in trial in the remaining 14 positive dogs with the help of their owners. They are also using insecticide-impregnated dog collars in order to protect the dogs from sand fly bites and prevent the disease from spreading to other dogs and possibly to humans.

In conclusion, our results demonstrate that CanL is widespread among dogs in western Turkey and the number of the clinical signs is very helpful for suspecting CanL but at least one serological and/or parasitological method should be performed for certain and differential diagnosis of the disease in the dogs. However, our findings showed that the one-third of the dogs in an endemic area of CanL could be asymptomatic.

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