

Seroprevalence of Canine Visceral Leishmaniasis in Kuşadası, Turkey

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Abstract: Human and canine visceral leishmaniasis cases have been reported from Kuşadası, a town in western Turkey, since 1993. In order to better understand the epidemiology of canine visceral leishmaniasis (CanVL) in the region, we aimed: (i) to determine the prevalence of CanVL in Kuşadası using a randomly selected dog population and (ii) to measure the effect of case control on the prevalence of the disease. In this study, all 109 dogs kept at the dog shelter of Kuşadası Municipality (reflecting random selectivity) were screened serologically using IFAT and rK39 ELISA in 1999. Ten dogs (9.1%) were seropositive or borderline in 1 of the 2 tests. Seropositive dogs (8) died spontaneously or were euthanized, while borderline dogs (2) underwent monthly serological examinations. One year later, in a second sampling, a total of 85 dogs were examined (27 previously tested and 58 new). Four dogs (4.7%) showed seropositivity. Seropositive dogs (2) were euthanized while borderline dogs (2) underwent monthly serological examinations. The reduction in the prevalence of the disease (from 9.1% to 4.7%) appears to be the result of culling CanVL positive dogs from the general population.

Key Words: Canine leishmaniasis, seroprevalence, Turkey

Kuşadası'nda Köpeklerde Visseral Leishmaniasisin Seroprevalansı

Özet: Kuşadası ilçesinden 1993 yılından beri insan ve son yıllarda da köpek visseral leishmaniasis olguları rapor edilmektedir. Bölgede köpeklerdeki visseral leishmaniasisin epidemiyolojinin daha iyi anlaşılabilmesi için; (i) Kuşadası'nda rastgele seçilmiş köpeklerde prevalansın saptanması, (ii) hastalığın prevalansı üzerine olgu kontrol çalışmasının etkisinin ölçülmesi amaçlanmıştır. Çalışmada, 1999 yılında belediye köpek evinden rastgele seçilen 109 köpek IFAT ve rK39 ELISA testleri ile serolojik olarak taranmıştır. Toplam 10 köpek (% 9,1) testlerin herhangi biriyle seropozitif veya sınırda pozitif olarak bulunmuştur. Bu köpeklerin 5 tanesi uyutulmuş, 3 tanesi kendiliğinden ölmüştür. Sınırdaki pozitif olan 2 köpek ise her ay serolojik olarak kontrol edilmiştir. Bir yıl sonra ikinci örneklemede 85 köpek (27'si geçen yıldan da test edilen ve 58'i yeni) incelenmiştir. Dört köpeğin (% 4,7) seropozitif veya sınırda pozitif olduğu saptanmıştır. İki seropozitif köpek uyutulurken, sınırdaki iki köpek yine aylık incelemeye alınmıştır. Hastalığın prevalansının % 9,1'den % 4,7'ye düşmesinin pozitif köpeklerin genel populasyondan uzaklaştırılmasına bağlı olduğu düşünülmüştür.

Anahtar Sözcükler: Leishmaniasis, köpek, seroprevalans, Türkiye

Introduction

The prevalence of canine visceral leishmaniasis (CanVL) in Mediterranean countries is reported to vary greatly from site to site with the percentage of infected dogs ranging from 1% to 37% (1,2). Even though dogs are thought to play an important role in human VL, no

direct correlation has been reported between the incidence of canine and human infection.

Although CanVL was first detected in the early 1950s by Yaşarol (3,4) in the cities of Bursa and İstanbul, knowledge about the epidemiology of this disease is limited in Turkey. In detailed screening carried out in the

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localities where human VL cases have been found in western Turkey, the mean seropositivity rate among dog populations was 5.3% (range 3.6%-25%). A significant percentage (85%) of the seropositive dogs were found to be positive by at least one of the following techniques: microscopical examination and culture or PCR of the lymph node aspiration material (5).

Human and canine VL cases have been reported from Kuşadası, a town in western Turkey. In Kuşadası, the dogs kept at the shelter belonging to the Municipality are brought in and adopted by people living in different areas of the town and thus represent random selectivity. The CanVL screening in this study was performed to determine the prevalence of the disease among the dog population in Kuşadası and to assess whether a canine-case control study would be helpful for controlling the disease in dog populations.

Materials and Methods

One hundred sixty-seven dogs were sampled at the Kuşadası Dog Shelter. All dogs were marked with numbered collars. Sampling was performed twice: before and after the transmission season (May/June 1999 and March/April 2000). One hundred nine dogs were tested in the first sampling, and 85 consisting of 27 previous and 58 new dogs, were tested in the second.

Five milliliters of venous blood was collected from each dog by brachial vein puncture. The samples were transported to the laboratory and kept at -20 °C until use.

Popliteal lymph node aspirations were obtained from a group of seropositive and seronegative dogs using 21G needles. Tissue aspirates were stained with Giemsa and examined by microscopy and inoculated into NNN culture.

An indirect fluorescent antibody test (IFAT) was carried out using promastigotes from local *L. infantum* strains (MON-1) obtained by mass culturing in RPMI-1640 containing 10% fetal calf serum (FCS). Promastigotes were harvested and washed 8 times in phosphate buffered saline (PBS). The IFAT was performed using standard procedures. A 2-fold serial dilution (1:16 to 1:16,384) of dog serum in PBS was used. A titer of 1:128 was considered positive for CanVL (6).

rK39 ELISA: polystyrene microtiter plates were coated by purified rK39 antigen at a concentration of 20 ng per well. ELISA was performed at 1:100 serum dilutions as described before using rabbit anti-dog IgG conjugated with horseradish peroxidase (Sigma) at a 1:1000 dilution and a 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tablet. The optical density was measured at 405 nm (7). The annual change in the prevalence was analyzed by the chi-square test.

Results

First Sampling

All 109 dogs in the shelter were screened serologically using IFAT and rK39 ELISA and a group of seropositive and seronegative dogs also underwent lymph node sampling for detecting Leishmania parasites.

Ten (9.1%) out of the 109 dogs were found to be either borderline or positive by serological tests. Popliteal lymph node samples from these 10 dogs and 9 seronegative dogs were aspirated. Leishmania parasites were detected in all the seropositive dogs (8) by microscopy and/or culture, while no Leishmania parasites were detected from the borderline or seronegative dogs.

Five out of the 10 dogs that had severe VL infection, showing almost all the clinical symptoms of CanVL, were euthanized by an authorized veterinary surgeon after obtaining permission from their owners and the town's animal rights organization. Another 3 of the 10 dogs with severe clinical signs such as intestinal bleeding and epistaxis died spontaneously within 2 weeks (Table). Two dogs had borderline titers in the IFAT while they were negative in rK39 ELISA test. These 2 dogs were collared using insecticide impregnated dog collars and followed up by monthly serological tests.

Second Sampling

A total of 85 dogs were examined (27 previously tested and 58 new dogs). Four (4.7%) out of the 85 dogs were found to be seropositive or borderline by all tests and/or lymph node aspiration. Seropositive dogs (2) were euthanized while borderline dogs (2) underwent monthly serological examinations (Table).

There was no statistical significance between the 2 percentages found in 1999 and 2000 ($P > 0.05$).

Table. The results of the dogs found to be VL seropositive in Kuşadası.

	Date	Dog No	Smear	rK39-ELISA	IFAT	Result
First Sampling	May/June 1999	1	POS	POS	2048	Euthanasia
		2	POS	POS	2048	Euthanasia
		3	POS	POS	2048	Spontaneous death
		4	POS	POS	2048	Spontaneous death
		5	POS	POS	2048	Euthanasia
		6	POS	POS	1024	Euthanasia
		7	POS	POS	1024	Euthanasia
		8	POS	POS	1024	Spontaneous death
		9	Neg	Borderline	128	Collared and followed
		10	Neg	Borderline	128	Collared and followed
Second Sampling	March/April 2000	11	POS	POS	512	Euthanasia
		12	POS	nd*	512	Euthanasia
		13	Nd	Borderline	256	Collared and followed
		14	Neg	Borderline	64	Collared and followed

*nd: not done

Discussion

Accurate measurements of the infection rate in dogs and of the distribution of active foci are essential if appropriate control strategies for VL are to be planned and executed. The determination of infection levels for canine populations depends on the evaluation of several parameters including disease symptoms, anti-leishmanial antibody titers, and microscopical detection of the parasite.

Leishmaniasis in dog populations receives utmost attention in areas where human VL is also endemic, although the role of infected dogs in the epidemiology of human VL is controversial. In some reports, infected dogs appear to have an impact on the epidemiology of human leishmaniasis, while in other studies no apparent direct correlation exists between the incidence of canine and human VL (8,9). The seropositivity rate during our first sampling (9.1%) was in concordance with our previous findings in Turkey and other Mediterranean countries (2,5). After the 1 year case-control program in this study, the seropositivity rate of the dog population decreased from 9.1% to 4.7%. This decrease is not statistically significant ($P > 0.05$) but, interestingly, before our research, 5 human VL patients had been recently reported in Kuşadası. However, no new human VL cases were reported in Kuşadası during the 2 years of our CanVL case-control program.

In this study, parasites were detected by microscopical examination in all serologically positive dogs (100% concordance) and no parasites were detected in any of the borderline dogs by microscopical examination or by culturing of parasites from lymph node aspirates. Failure to detect parasites in lymph node aspirates in borderline dogs may be due to either very low parasite levels or the dog may have had a subclinical infection in the past. Therefore, a microscopical examination and/or culturing are not as reliable as gold standards. More sensitive parasitological examinations such as PCR and serological assays using different antigens together are recommended for a more reliable diagnosis of the disease.

In conclusion, culling the CanVL cases from the general dog population appears to be an effective approach for interrupting the transmission of the Leishmania parasite in CanVL endemic regions. Based on our results, we recommend that public health officials in endemic areas screen the dog population serologically every year before the transmission season (just before summer). Lastly, further research is needed to develop effective treatment methods and/or a vaccine for canine leishmaniasis.

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