EMERGENCE OF VISCERAL LEISHMANIASIS IN CENTRAL ISRAEL

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Abstract. In 1994–1995, a child and five dogs from villages located between Jerusalem and Tel-Aviv, Israel were diagnosed with visceral leishmaniasis (VL). Based on these findings, the distribution of VL in domestic and wild canids in central Israel was examined. In the two villages where canine index cases were identified, a substantial proportion (11.5%, 14 of 122) of the dogs examined were seropositive. However, the rate of infection in five neighboring villages was only 1% (1 of 99). Parasites were cultured from 92% (12 of 13) of the seropositive dogs biopsied and the strains were characterized as Leishmania infantum by a clamped polymorphic–polymerase chain reaction, monoclonal antibodies, and/or excreted factor serology. The discovery of VL close to major urban centers is an important public health issue. The disease appears to have emerged recently in this area, and it is unclear whether the parasite was re-introduced or was continuously present at low levels in this region. The presence of seropositive wild canids, jackals (7.6%, 4 of 53) and red foxes (5%, 1 of 20), in central Israel, and the reappearance of the jackal population after near extinction suggests that wild canids may play a role in spreading this disease.

Human and canine visceral leishmaniasis (VL), caused by the parasite Leishmania infantum, have been described in most countries bordering the Mediterranean Sea and throughout the Middle East. In the former countries, the dog is the principle peridomestic reservoir for VL and disease prevalence ranges from < 1% to 37%. In addition to dogs, other canids often found in the vicinity of humans, such as jackals (Canis aureus) and red foxes (Vulpes vulpes), are potential feral reservoirs for L. infantum. Visceral leishmaniasis in the jackal has been described in Iran, Iraq, and Kazakhstan, while VL in the red fox has been reported from France, Portugal, and Italy.

Both cutaneous and visceral disease are endemic to Israel. Cutaneous leishmaniasis, the mild self-curing form of the disease, is common in eastern and southern Israel along the Dead Sea Rift Valley and the Negev Desert. Fatal VL, though less prevalent, is endemic to northern Israel where both human and canine disease occurs. For more than 30 years prior to 1994, no autochthonous cases of VL were diagnosed in central Israel, the area between Jerusalem and Tel Aviv. However, in 1994 a dog from Nataf, 11 km west of Jerusalem, was referred for dermatologic consultation to the School of Veterinary Medicine in Rehovot. Promastigotes, typed as L. infantum, were isolated from the dog. In the same year a child from Matityahu, a village 22 km north-west of Jerusalem, was diagnosed with VL. In 1995, four additional dogs from the same general area were diagnosed with VL. Based on these index cases, we initiated a preliminary study on the distribution of canine VL in central Israel.

MATERIALS AND METHODS

Dogs. Household dogs from two villages, Nili (62) and Nataf (60), where the disease was initially identified (Figure 1) were examined for clinical symptoms of VL. Dogs were examined for skin lesions, lymphadenomegaly, and poor body condition, the last of which was indicated by weight loss. Blood was taken by cephalic venipuncture for determination of anti-leishmanial antibody levels by an ELISA.

In addition, dogs from five villages, Hashmonaim, Mevo-Horon, Matityahu, Shilat, and Na’ale, in a 10-km radius around the index cases (Figure 1) were also screened for VL by the above methods. Seropositive dogs were re-visited. Lymph node and splenic aspirates were cultured for parasites.

Jackals and foxes. Whole blood samples were collected by cardiac puncture and/or cephalic venipuncture from 24 jackals and 20 foxes shot or trapped in central Israel (Figure 1) as part of an experiment on oral rabies vaccination conducted by the Nature Reserves Authorities and the Ministry of Agriculture.

Additional sera were collected from foxes (15) and jackals (39) shot by Nature Reserves Authorities wardens as part of a government policy to limit the spread of rabies in areas where disease outbreaks have occurred (Figure 1). All bloods were allowed to clot and the serum was collected by centrifugation (10 min at 3,000 rpm) for testing by ELISA.

Enzyme-linked immunosorbent assay. Serum anti-leishmanial antibodies were determined by ELISA, using crude leishmanial antigen, essentially as previously described. All dog sera, tested at two dilutions (1:100 and 1:1,000), were incubated with antigen coated plates for 1 hr at 37°C. The plates were then washed with 0.1% Tween 20 in 50 mM phosphate-buffered saline (PBS), pH 7.2, and incubated with Protein A conjugated to horseradish peroxidase (Zymed Laboratories, Inc., San Francisco, CA) for 1 hr at 37°C. Excess conjugate was removed by extensive washing in PBS-Tween and the plates were developed by addition of the substrate 2,2′-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) (Boehringer Mannheim, Mannheim, Germany). Each plate was read when the absorbance (λ = 405 nm) of the positive canine reference serum reached a value between 0.95 and 1.0. A titration of positive and negative reference dog sera was included on each plate to monitor interassay variation. A sample was considered positive if the optical density was 2.6 times higher than the standard deviation of the control group.

Jackal and fox sera were tested by the ELISA described in this report. In addition, these sera were also examined using rabbit anti-dog IgG (heavy plus light chain) conjugated to horseradish peroxidase (1:5,000 dilution; Jackson Labo-
ratories, Inc., West Grove, PA) instead of the Protein A conjugate.

**Parasites.** Biopsy samples from spleens and/or lymph nodes were cultured on NNN slants overlaid with Schneider’s *Drosophila* medium and/or on Evan’s semi-solid medium. Promastigotes from positive isolates were subcultured in Schneider’s *Drosophila* medium containing 10% fetal calf serum and used for strain characterization. Strains were characterized by one or more techniques including excreted factor serology, species-specific monoclonal antibodies, and/or a clamped polymorphic–polymerase chain reaction (CP-PCR, Eisenberger CL, Jaffe CL, unpublished data).

**RESULTS**

**Dogs.** In the last three months of 1995, four household dogs were referred to the School of Veterinary Medicine by local veterinarians and diagnosed with VL. Two dogs came from Nataf, the site of the original canine index case. The third case was from Nili, a village 26 km northwest of Jerusalem and 4 km north of Matityahu in the Modi’in Region where the human index case was diagnosed. The fourth dog was from the Sataf Nature Reserve 2 km west of Jerusalem (Figure 1). During February and March 1996, the majority of the canine population in Nataf (60 of 65, 92%) and Nili (62 of 66, 94%) was screened for clinical signs of VL and for serum anti-leishmanial antibodies. The prevalence of seropositive dogs in Nataf and Nili was found to be 10% (6 of 60) and 12.9% (8 of 62), respectively. All of the seropositive dogs (14) were re-examined for clinical signs of disease and biopsied for parasites. Twenty-eight percent (4 of 14) of the dogs showed no clinical signs of disease. Of the symptomatic dogs, 36% (5 of 14) had mild exfoliative dermatitis with enlargement of some external lymph nodes and 36% (5 of 14) showed considerable weight loss, dermatitis, and generalized enlargement of lymph nodes. Promastigotes were cultured from 92% (12 of 13) of the animals biopsied. A single seropositive dog died between the first visit and the re-examination, and did not undergo a biopsy.

Dogs (99) in five additional villages (Hashmonaim, Mevo Horon, Matityahu, Shilat, and Na’ale) from the Modi’in Region located approximately within a 10-km radius of Nili were tested for VL. This included > 95% of the dogs in each village, except for Hashmonaim where approximately > 80% of the dogs were examined. Only one dog, from Na’ale (2.5 km east of Nili), was seropositive.

An additional two dogs with VL from central Israel were identified by passive case detection during 1996. These sick dogs came from the city of Rishon LeZion and the village of Aviezer (Figure 1). Both dogs were symptomatic and seropositive, and promastigotes were cultured from lymph node and spleen tissue biopsies.

**Jackals and foxes.** The ELISA on serum from jackals or foxes was carried out with anti-dog IgG horseradish peroxidase conjugates as the second antibody. This assay was more sensitive for measuring anti-leishmanial antibodies in jackals and foxes than an equivalent assay using Protein A–horseradish peroxidase conjugates. A total of 63 serum samples were collected from jackals for analysis of anti-leishmanial antibodies. The majority of the sera (53) were obtained from jackals shot or trapped in the Modi’in Region, 34 from a land fill 8 km west of Matityahu and 19 from locations near villages (< 10 km) where canine or human VL was detected (Figure 1). Control sera (9) were collected 30 km southwest of the new VL focus, near Beit Guvrin (Figure 1). As yet, no cases of VL have been reported from that area.

Approximately 7.6% (4 of 53) of the jackal sera collected in the Modi’in Region (Figure 2) were positive. Indeed, the mean absorbance (0.2133) for sera from the Modi’in Region was significantly higher than that found for the negative control region (mean = 0.0733; P < 0.0001, by two tailed t-test), supporting the supposition that a large number of jackals in the former region are infected with *L. infantum*.

A total of 35 sera samples from foxes were examined for anti-leishmanial antibodies. The majority of the sera (20 of 35) came from the coastal region 18 km south of Tel Aviv (Figure 1). This is located 30 km west of the Modi’in region, where most of the jackal and canine studies were carried out. However, one isolated case of canine VL was recently diagnosed in Rishon LeZion, a city adjacent to this area. The additional samples originated from foxes shot within the Modi’in Region (2) and from animals shot during a rabies outbreak in the Dead Sea Rift Valley, north of Eilat (13). The latter sera were from an area endemic for human cutaneous leishmaniasis. Neither human nor canine VL has been reported in the regions and these sera served as controls.

One serum from a fox in the coastal region and in the Rift Valley was seropositive (mean + 2.6 SD; P > 99%). All the other sera were negative. The means for the two regions were different (P = 0.02).

**Parasites.** Promastigotes were successfully cultured from 14 (87.5%) of 16 seropositive dogs that were biopsied. One seropositive dog did not undergo a biopsy. All the isolates were characterized by excreted factor serotyping and had a B2 subsertype typical of *L. donovani sensu lato*. Three strains were also examined by ELISA using species-specific monoclonal antibodies for *L. donovani* (D2), *L. tropica* (T11 and T13), and *L. major* (T1). All these isolates reacted only with D2, typical of the *L. donovani* complex. Finally, the
isolates were examined by CP-PCR. The PCR was carried out using a GC-rich oligonucleotide primer, based on the DNA sequence of the *L. chagasi* homolog for the *L. major* gene B/C intergenic region and an oligonucleotide primer overlapping with the T3 region of the pBluescript SK vector (Stratagene, La Jolla, CA) (Figure 3). Following amplification by the PCR, the pattern observed for each of the Israeli isolates was examined and compared with DNA from World Health Organization reference strains for *Leishmania major* (*Lm*), *L. tropica* (*Lt*), and *L. infantum* (*Li*). The CP-PCR, 30 cycles using template DNA (10 ng), was carried out using a specific primer for *Leishmania* taken from the *L. chagasi* gene B/C intergenic region and a nonspecific primer overlapping with the T3 region of the pBluescript SK vector. The reaction products were separated by electrophoresis on 3% agarose gels and stained with ethidium bromide. Values on the left are in basepairs.

**FIGURE 2.** Serologic survey of anti-leishmanial antibodies in foxes and jackals. An assay (ELISA) with crude *Leishmania donovani* antigen was carried out using sera collected in central and southern Israel. Anti-leishmanial antibodies were measured at serum dilutions of 1:100 and 1:1,000, using an anti-dog IgG horseradish peroxidase conjugate. Sera was collected from foxes in the Mediterranean coastal region (COAST) and the Dead Sea Rift Valley near Eilat (RIFT). Jackal sera were collected in and around Modi'in and Nataf, where canine visceral leishmaniasis was found (MODIIN) and from an area apparently free of canine disease at this time (NEG). The horizontal bars show the mean absorbance of each group tested at a 1:1,000 dilution.

**FIGURE 3.** Identification of leishmanial strains isolated from dogs in central Israel by the clamped polymorphic–polymerase chain reaction (CP-PCR). The DNA purified from seven different dog isolates was examined and compared with DNA from World Health Organization reference strains for *Leishmania major* (*Lm*), *L. tropica* (*Lt*), and *L. infantum* (*Li*). The CP-PCR, 30 cycles using template DNA (10 ng), was carried out using a specific primer for *Leishmania* taken from the *L. chagasi* gene B/C intergenic region and a nonspecific primer overlapping with the T3 region of the pBluescript SK vector. The reaction products were separated by electrophoresis on 3% agarose gels and stained with ethidium bromide. Values on the left are in basepairs.

**DISCUSSION**

Continuous sporadic human VL has been documented over the last 40 years in the Galilee Region of northern Israel. More recently, canine VL was also identified in this same region. However, for more than 30 years no documented cases of either human or canine disease have been reported from central Israel. This study shows that canine VL is prevalent in the Judean foothills between Jerusalem and Tel Aviv. The discovery of VL in central Israel indicates that VL is a common infection in the newly described focus. In common with other endemic areas throughout the Mediterranean Region, this disease is highly focal in nature with prevalence varying markedly among villages located in close proximity to each other. In the two villages (Nili and Nataf) where the canine index cases were identified, a substantial proportion (11.5%, 14 of 122) of dogs examined were found to be infected. However, among the five villages sampled that neighbor Nili, the rate of infection was only 1% (1 of 99). The reasons behind the focal nature of this disease are unknown and may depend on specific ecologic factors.

The prevalence of canine VL in the Mediterranean region varies considerably from about 1–37% and appears to fluctuate over time, with the infection rates increasing in some foci and disappearing in others. In one earlier study on canine VL in northern Israel, 5% of the dogs at Wadi Hamam near the Sea of Galilee were seropositive. However, all estimates regarding the prevalence of VL are probably underestimates, since infected dogs can remain seronegative and asymptomatic for many months prior to the appearance of anti-leishmanial antibodies and clinical symptoms of disease.

The role of jackals and foxes in the epidemiology of canine and human VL in Israel can not be evaluated solely on
the basis of this study. However, the serologic evidence presented here indicates that a considerable proportion of the jackal population living in the central Israeli focus are probably infected. Wild canid populations in Israel came close to extinction during the 1950s and 1960s as a result of a rabies control program that included the elimination of potential canid reservoirs.18 Recent annual counts of wild canids (Eli Shay, Nature Reserves Authorities, unpublished data) in central Israel have shown a dramatic increase in the jackal population during the last decade, and a progressive incursion into southern Israel. These observations, and the presence of seropositive animals in the newly described VL focus, suggest that wild canids may play a role in the spread of VL from northern to central Israel. Peridomestic transmission from jackals to people and/or dogs via sand flies can take place either when wild canids enter villages to forage for food or when the household dogs range in the extensive noncultivated areas surrounding the villages.

Local awareness of residents, veterinarians, and health professionals is important if emergence of this disease to be monitored efficiently. The index case in Nili was brought in for veterinary inspection after its owners read about canine VL in a local newspaper. Four additional dogs were referred to the us by local veterinarians, following updates about this disease either in professional journals or by direct contact. Diagnosis of a sick dog by passive case detection often resulted in the identification of additional infected dogs in the same village by active case detection. Additional epidemiologic studies are needed to monitor the spread of VL in central Israel and other parts of the country. These studies should also be useful in identifying the factors leading to the emergence of VL.

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