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FREE-LIVING JACKALS *(Canis mesomelas)* - POTENTIAL RESERVOIR HOSTS FOR *Ehrlichia canis* IN KENYA [1] [2]

JENNIFER E. PRICE and LARS H. KARSTAD

Abstract: Using a modified cell culture test, *Ehrlichia canis* was found in eight of 16 free-living jackals *(Canis mesomelas)* and 14 of 31 dogs owned by pastoral communities in the same areas of Kenya. Two cross-bred puppies inoculated with blood from infected jackals developed mild, transient clinical disease, and *E. canis* was recovered from the puppies. Tick species found on the jackals were similar to those found on the infected dogs. *Ehrlichia canis* was not found in eight spotted hyaenas *(Crocuta crocuta)* from these areas.

INTRODUCTION

Canine ehrlichiosis, caused by the rickettsia, *Ehrlichia canis,* has been described in Kenya [2,10] and other parts of the world. [4,16,17] Various breeds of dogs have been naturally and experimentally infected by ticks and by inoculation of blood from infected dogs. [9,11,17] Experimental infections with *E. canis* were studied in the silver-backed jackal *(Canis mesomelas),* [14] the coyote *(Canis latrans),* [5] the American red fox *(Vulpes fulva)* and gray fox *(Urocyon cinereoargenteus).* [1] A fatal case of canine ehrlichiosis was reported in a silver-backed jackal after it had been exposed to ticks in the Veterinary Hospital at Onderstepoort (South Africa) [13] and an epizootic of ehrlichiosis has been described in wolves and wolf-dog crosses. [7] *Rhipicephalus sanguineus* is the natural vector of *E. canis* [3] and transmits the disease transstadially. [6] Infected ticks have been shown to transmit *E. canis* to susceptible dogs for 155 days after engorgement on dogs in the acute phase of ehrlichiosis. [12]

MATERIALS AND METHODS

Animals

Jackals. Sixteen adult silver-backed jackals were studied: nine were shot just outside the Mara Reserve in Kenya, one was found with a suspected hip fracture in Nairobi National Park, three were live-trapped at Athi River and three were captured on the Loita Plains, Narok District, by drug immobilization using 200 mg ketamine hydrochloride [3] in projectile syringes.

Jackals captured at Athi River (Jackals 11, 12 and 13), on the Loita Plains (Jackals 29, 30 and 31) and in Nairobi National Park (Jackal 4) were anaesthetized with ketamine hydrochloride, using 25 mg/kg and then brought to the clinic. Jackals 12, 29 and 31 were held in captivity.

[1] From the Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, Kenya and the Veterinary Research Laboratories, Kabete, Kenya. Research conducted under a cooperative project of the Government of Kenya and the Canadian International Development Agency, with the International Development Research Centre as the implementing agency. The opinions expressed are those of the authors, not necessarily those of the supporting organizations.

[2] Published with the approval of the Director of Veterinary Services, Kenya.

[3] Ketalar, Laboratories Parke-Davis, S.A.W. Madrin - Spain.

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Hyaenas. Eight spotted hyaenas *(Crocuta crocuta)* were used in this study. These were captured in the Mara area, Narok District.

Domestic dogs. Eight Masai owned domestic dogs from Narok District, four Masai owned domestic dogs from Konza area, Kajiado District and 11 Turkana owned domestic dogs from Kakuma, Turkana District were used.

Since a high prevalence of canine ehrlichiosis occurs in Kenya, it was difficult to obtain uninfected dogs. Therefore, uninfected puppies were reared by whelping a bitch in tick-proof kennels.

Pups from a pregnant bitch, shown to be negative for *E. canis* by the modified cell culture test, were tested periodically for *E. canis* using the cell culture test and were consistently found to be negative. Three of these three-month old puppies were used in this study.

Samples collected

Blood. Blood from all the jackals, hyaenas and domestic dogs were tested for *E. canis* organisms by the modified cell culture test. This test was modified from that originally described by Nyindo *et al.*[15] by using 10 ml of heparinized blood from each animal instead of 50 ml; moreover, culture medium was not added to the Leighton tubes during incubation, as originally described by Nyindo *et al.*[15]

In the animals that were bled in the field, 10 ml of whole blood was drawn into a sterile heparin-coated syringe. A second needle was fitted and bent at right-angles and the syringe taped to a convenient tree. The syringe was not moved until the blood had sedimented. Two millilitres of leucocyte-rich plasma were transferred through the bent needle into sterile Leighton tissue culture tubes. After 2 to 4 days incubation at 37 C, or in the field at ambient temperatures below 37 C but as closely as possible approaching that temperature, the coverslips within the Leighton tubes were washed with saline and the cells fixed with methanol. The Leighton tubes with the coverslips could then be transported to the clinic, where the cells were stained with Giemsa and then microscopically examined for *E. canis* morulae as described by Nyindo *et al.*[15]

Since jackal blood sediments very slowly, an additional 20 ml of heparinized blood was taken from those jackals that were bled in the clinic. The blood was placed in a sterile universal bottle and centrifuged at 150 G for 10 min. The plasma was then transferred to Leighton tubes and the culture test carried out as previously described.

Infected leucocytes from jackals 29 and 30 were established in culture and 5 ml were inoculated intravenously into 2 three-month old, cross-bred puppies. A litter mate was used as an uninfected control. Clinical signs and haematological parameters were monitored in these puppies. When the puppies showed clinical signs indicative of ehrlichiosis, blood was taken for the modified cell culture test.

Ticks. Ticks on the jackals and domestic dogs were collected and kept on ice until they were identified at the clinic.

RESULTS

Blood samples

Jackals. Eight of the 16 (50%) free-living jackals tested were shown to harbour *E. canis*. This number may have been higher, but difficulties were encountered using the test under field conditions. Jackal blood took 3 to 5 hours to sediment, even in animals harbouring *E. canis*. Many samples were heavily contaminated with bacteria or had microfilariae, interferring with the test. There was no morphological difference between cells collected by slow centrifugation and those collected by the sedimentation technique. In samples from animals infected with microfilariae, the microfilariae prevented cells from settling on the coverslips in the

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Leighton tubes. Microfilariae were identified as *Dipetalonema reconditum* (J.H. Arundel, pers. comm.).

Hyaenas. The blood from all eight hyaenas which was examined for *E. canis* was negative. However, the blood sedimented in 15 to 20 min.

Dogs. The blood specimens from 6 of the 8 domestic dogs from Narok District were positive for *E. canis*. All four domestic dogs from Kajiado District were shown to harbour *E. canis* while only 4 of the 11 dogs examined from Turkana District were positive. The blood from these infected domestic dogs sedimented within 60 min.

The two puppies inoculated with leucocyte culture material from jackals 29 and 30 showed mild clinical disease. Dog A, inoculated with cells from jackal 30, had a biphasic temperature rise to 39.5 C on Day 4 and 10 days later to 39.3 C. Dog B, inoculated with cells from jackal 29, had a temperature of 39.8 C on Day 3. Transient anorexia and depression were seen in both dogs and Dog A had splenomegaly. However, these clinical signs were very mild and the puppies were clinically normal after three weeks. Seven to 9 days after inoculation Dog B showed mild transient anaemia and leucopenia. On Days 9 and 5 post inoculation, Dog A and Dog B were shown by the cell culture test to harbour *E. canis*.

TABLE 1. Tick species found on 12 free-living jackals and eight domestic dogs in Narok District and two dogs in Turkana District.

Tick Species	Jackals	Dogs
Rhipicephalus sanguineus	5/12	5/10
Rhipicephalus simus	7/12	2/10
Rhipicephalus appendiculatus	0/12	3/10
Rhipicephalus pulchellus	0/12	1/10
Amblyomma nymphs	6/12	1/10
Amblyomma variegatum	1/12	2/10
Haemaphysalis leachii	11/12	5/10

Ticks. The tick species found on the jackals from Narok and Kajiado Districts are given in Table 1. *Haemaphysalis leachii* was the most commonly encountered tick, being present on 11 of the 12 jackals that harboured ticks. *Rhipicephalus simus* was found on seven jackals while *R. sanguineus* was only found on five. In general, domestic dogs harboured fewer ticks than free-living jackals. Only 5 of the 10 dogs examined had *H. leachii* and five dogs had *R. sanguineus*.

DISCUSSION

Silver-backed jackals have been experimentally infected with *E. canis*[14] and one jackal was infected by exposure to ticks.[13] This study shows the presence of natural infection with *E. canis* in free-living jackals. Domestic dogs in pastoral communities in Narok, Kajiado and Turkana Districts also were shown to harbour *E. canis*. However, hyaenas from the same areas as the jackals and domestic dogs, tested with the same technique, did not harbour *E. canis*. This supports the opinion that *E. canis*, even in free-living animals, is restricted to Canidae.[8]

Clinical disease was not evident in captured jackals in this study and infection was identified only by *in vitro* culture. The mild transient disease observed in puppies inoculated with cells from these cultures was similar to that seen in cross-bred puppies infected with blood from domestic dogs in the acute phase of ehrlichiosis. The very mild disease in these puppies may be due to the low infectivity of the culture material.

The sensitivity of the modified cell culture method has been tested on 91 working dogs from the police and armed forces. These dogs were clinically normal and did not develop clinical signs of ehrlichiosis even though they were sub-

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jected to strenuous exercise or work. The modified cell culture test, however, showed that 71 of these dogs were positive for *E. canis*.

Since jackal blood had to be left in syringes for up to 5 h before sedimentation was complete, bacterial contamination frequently occurred. To avoid contamination, jackal blood was centrifuged very slowly and then cultured in Leighton tubes as described. Centrifugation did not have any apparent adverse effect on the mononuclear cells. While microfilariae of *D. reconditum* prevented cells from settling on the coverslip, this could not be avoided. Apart from these difficulties, the cell culture test worked well under field conditions. Environmental temperatures were apparently sufficient for *E. canis* to develop and multiply inside the cytoplasm of the mononuclear cells on the coverslips. Washing and fixing the cells on the coverslips within the Leighton tubes enabled the cultures to be transported back to the clinic for microscopic examination.

Rhipicephalus sanguineus is the only proven vector of *E. canis* in domestic dogs.[4,6] Pastoral domestic dogs generally had fewer ticks than the jackals. *R. sanguineus* and *H. leachii* were equally common on these dogs but a greater number of jackals harboured *H. leachii* than *R. sanguineus*. Although *E. canis* has now been confirmed in free-living jackals, the vector has to be identified. Further work on tick transmission of *E. canis* to jackals should be carried out, using both *R. sanguineus* and *H. leachii*.

These studies indicate that domestic dogs are susceptible to infection with *E. canis* from jackals and provides further evidence that the infection is confined to the Canidae. Since the same species of ticks were found on both jackals and pastoral domestic dogs, cross infestation may occur where the two species intermingle. Jackals thus may act as a reservoir host for *E. canis* in Kenya.

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