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Parasitological and Serological Survey of Domestic Goats for Leishmaniosis in Baringo District, Kenya

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ABSTRACT

A parasitological and serological study of goats was conducted in May, 1993 in Baringo District, Kenya, to determine the presence of *Leishmania* parasites and circulating *Leishmania*-specific IgG antibodies. A total of 102 goats were sampled for the presence of natural infections with *Leishmania* parasites at six different households. Venous blood and bone marrow samples, and saline aspirates from lymph node and sub-cutaneous tissues were drawn from each animal and cultured in NNN diphasic media in the laboratory. In addition, sera from goats and 23 human occupants of the households were assayed for the presence of *L. donovani*-specific and *L. major*-specific IgG antibodies, using an enzyme-linked immunosorbent assay (ELISA). No flagellate protozoans were cultured from the samples. However, *L. donovani*-specific IgG antibodies were detected from two goats (2.0%) and six humans (26.1%). These data indicate that domestic goats are exposed to *Leishmania* parasites and produce detectable antibody responses. However, this is viewed as an accidental infection that does not develop into active disease. Our data does not support the implication that goats are potential reservoirs for human leishmaniasis.

INTRODUCTION

Visceral leishmaniasis (kala-azar) caused by *Leishmania donovani* is endemic in the lowland areas of Baringo District, Rift Valley, Kenya. Historically, a number of investigators have focused intensive research efforts on identifying animal reservoirs of visceral leishmaniasis in Kenya. The sporadic nature of this disease in endemic areas strongly suggest and animal reservoir. However, no domestic or wild mammal has been confirmed as the reservoir of visceral leishmaniasis in East Africa. Over 30 years ago the following question was asked of the scientific community, "Is there an animal reservoir of kala-azar in Kenya?" (Heisch 1963). This question continues to puzzle researchers even today.

In Kenya, isolations of *Leishmania* parasites, suspected as being *L. donovani*, have been reported from a variety of domestic and wild mammals (Abranches 1989; Ngoka and Mutinga 1978). The list of *Leishmania* isolations and suspected reservoirs of visceral leishmaniasis include dogs (Ngoka and Mutinga 1977; Mutinga et al. 1980) mongooses and a genet cat (Mutinga et al. 1982), a ground squirrel (Heisch 1957a) and gerbills (Heisch 1956b). However, these early studies did not characterize the isolates and often presumed the parasite species based on their organ specificity. In addition, these early researchers collected extremely low numbers of infected animals (often a single animal) making the assumption of the reservoir status of the animal species suspect. These early observations require confirmation using modern biochemical techniques to positively identify the parasite species.

It has been suggested that domestic sheep in South Africa (Van Der Lugt et al. 1992) and goats in Kenya (Mutinga et al. 1988; Mutinga et al. 1989) may act as reservoirs for human leishmaniasis. However, parasites collected during these early studies were not positively identified as *Leishmania* or biochemically characterised. The determination that the parasites isolated in South Africa were *Leishmania* sp. was based on the morphological characteristics of the basophilic bodies seen in tissue sections. Leishmanial parasites were cultured from four of 457 goats from West Pokot, Kenya (Mutinga et al. 1988). Recently, isoenzyme studies of one isolate from a goat in Kenya suggests *L. aethiopica* as the strain responsible for the infection (Williams et al. 1991). Unfortunately, the authors do not indicate what geographic area of Kenya the goat came from.

In Baringo District, Kenya, goats live in close proximity to man in areas of endemic visceral and cutaneous leishmaniasis and graze in close proximity to termite hills which are known sand fly resting sites. These animals are usually confined at night in an enclosure adjacent to households. This study investigated the presence of *Leishmania* in domestic goats, and *L. donovani*-specific and *L. major*-specific IgG antibodies from goats and humans, which would shed more

light on their potential role as reservoirs of human leishmaniasis.

MATERIALS AND METHODS

Study sites were chosen from within 10 km of Marigat town (0° 30' N Lat., 36° 0' E Long) in the Rift Valley about 250 km NW of Nairobi. The area is a semi-arid alluvial plain characterised by rolling hills covered with thorns, weeds, large termite hills and thick Acacia brush. Goats are the predominant livestock animal present in the area.

The six households where goats and humans were sampled were selected E/83, NLB-144) antigen. All reagents were used in the amount of 50 μl per well. The plates were washed three times, blocked with 5% bovine serum albumin in phosphate buffered saline containing 0.05% Tween 20 (PBS Tween) and coated with goat (1:3,200), sheep (1:800) or human (1:200) serum in PBS Tween. After one hour incubation and three more washings, anti-goat (1:3,200) or anti-human (1:500) IgG horseradish peroxidase (HRP) conjugate (Kirkgaard & Perry Laboratories, Gaithersburg, MD, USA) was added. Substrate 3,3',5,5'-Tetramethylbenzidine, (TMB Microwell System, Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) was added, then the reaction was stopped by adding 50 μl 1N H_3PO_4 within 15 minutes and the absorbance read at 450 nm wavelength. The mean of triplicate absorbance values were considered positive if they exceeded twice the mean values of known negative control sera.

RESULTS

A total of 102 goats and seven sheep were parasitologically sampled for the presence of *Leishmania* parasites. In the laboratory, 545 culture tubes containing samples were microsopically observed daily for the presence of flagellate protozoans. Fourteen tubes (2.6%) were contaminated with fungal growth. No flagellate protozoans were cultured from blood, bone marrow, saline aspirates from lymph node, or sub-cutaneous tissue aspirates. No cutaneous lesions suggestive of cutaneous leishmaniasis were observed on the goats.

Leishmania donovani-specific IgG antibodies were detected from seven goats (6.8%) and 11 humans (47.8%), and *L. major*-specific IgG antibodies were detected from two goats (2.0%) and six humans (26.1%) (Table 1). Of the *L. donovani*-positive sera, one male goat from household 3 and four human sera (two males and two females) from different households (1,2,5,6) also reacted with *L. major* antigens. Six male and five female humans had detectable *L. donovani*-specific antibody titers. Five of the 11 *L. donovani* seropositive humans had no history of prior infection with *Leishmania*. Of the seven goats that were found to be serologically positive, four were male and three were female. *Leishmania*

serologically positive humans were detected from all six households sampled. Serologically positive goats were detected from all study sites except household 4.

Table 1 Goats and humans serologically positive for *Leishmania*-specific antibodies.

	Goats			Humans		
House#	#Tested	L.d.+	L.m.+	#Tested	L.d.+	L.m.+
1	10	1	0	7	1	2
2	6	1	0	4	2	1
3	19	2	2	5	2	0
4	19	0	0	3	2	1
5	12	1	0	2	2	1
6	36	2	0	2	2	1
Totals	102	7	2	23	11	6
%Positive		6.9	2.0		47.8	26.1

L.d.+ = *Leishmania donovani* IgG antibody response
L.m.+ = *Leishmania major* IgG antibody response

DISCUSSION

The only records of *Leishmania* parasites isolated from domestic goats in Kenya are from West Pokot (Mutinga et al. 1988) and Transmara, Narok District (Mutinga et al. 1989). Recently, one additional parasite isolation from a goat in Kenya was tentatively identified as *L. aethiopica*, using isoenzyme studies (Williams et al. 1991). The epidemiological implications of these findings have yet to be elucidated and the suggestion that goats are reservoirs of leishmaniosis is still in question.

The current research sampled a large number of goats in Baringo District, Kenya, an endemic area for both cutaneous and visceral leishmaniasis, and found no *Leishmania*-infected animals. If goats were an important reservoir for either of these diseases, it would be reasonable to assume that some percentage of these animals would be culture positive for *Leishmania* parasites. Goats possess many of the characteristics of a "good" reservoir for leishmaniasis as previously defined (Bray 1982). Goats are in constant contact with sandflies and humans, they rest and breed in a climatic situation suitable for the feeding of the sandfly vector and are a major blood meal source for the vector sandfly (Ngumbi et al. 1992). Previous research in our laboratory revealed that high proportions of Phlebotomus martini Parrot, the vector of visceral leishmaniasis in Baringo

District, feeds on both human and goat blood, indicating repeated exposure of the goats to *Leishmania* antigens (Ngumbi et al. 1992) However, goats do not appear to continuosly present the disease organisms to the sandfly vector as evidenced by the data in this study and goats do not seem particularly susceptible to the disease organisms (Anjili et al. 1994).

It was recently reported that goats can become experimentally infected with *L. major* through needle inoculation of culture-derived promastigotes, but not by bites of infected *Phlebotomus duboscqi* sandflies (Anjili et al. 1994), and the presence of *Leishmania* at the site of inoculation was demonstrated in aspirate cultures at 28 and 42 days post-inoculation from the four goats that were needle-challenged. This same breed of goat is resistant to infection with *L. donovani* (Strain MHOM/SD/62/1-B/NLB-361) when infected intravenously with as many as 1x108 stationary phase promastigotes (LLR & AMS, unpublished data). No parasites were demonstrated in aspirate cultures of blood, bone marrow, lymph node or subcutaneous tissues at 7, 14, 30, 60 and 90 days post-inoculation. However, detectable levels of *L. donovani*-specific IgG antibodies were observed 60 days post-inoculation.

A small percentage of goats (6.8% and 2.0%) and a large percentage of humans samples in this study (47.8 and 26.1%) were serologically positive for *L. donovani*-specific and *L. major*-specific IgG antibodies, respectively. This is consistent with previous research that indicates that both *L. donovani* and *L. major* occur in this area. These serological data indicate that the goats and humans were infected at sometime in the past with *Leishmania* parasites and had a significant antibody response to the exposure, or that they have a current infection that is not detectable by the methods used in this study.

The fact that humans and goats at the same household are serologically positve for *Leishmania* raises an interesting question. Are the animals getting infected from the humans through the bite of infected sandflies or vice versa, or are these infections related?. This could in part explain serological positivity of goats sampled in Baringo District. During an early visceral leishmaniasis epidemiological study in Kitui District, Kenya, a positive correlation of the disease incidence and the presence of domestic animals was observed (Southgate and Oriedo 1962). However, researchers conducting a similar study in Meru District concluded that livestock did not seem to have a significant influence on visceral leishmaniasis incidence (Wijers and Mwangi 1966).

This research does not confirm the suggestion that goats can act as reservoirs for leishmaniasis and transmit this disease to humans in Kenya. Whether the presence of detectable levels of *Leishmania*-specific antibodies in goats means they may be transient reservoirs of leishmaniasis remains

undetermined. These results strongly suggest that visceral leishmaniasis in Kenya is anthropogenetic and not zoonotic. Our findings do indicate however the inclusion of goats in future sero-epidemiological studies in endemic areas. They may serve as "sentinel" animals because of the correlation of *Leishmania* seropositivity in goats and human subjects.

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REFERENCES

Abranches, P. 1989. Reservoirs of visceral leishmaniasis. 163, 61-70. *In:* Leishmaniasis: The current status and new strategies for control. NATO ASI series. Hart D.T. (Ed) Life Sciences, Plenum Press, New York.

Anjili, C. O., Olobo, J. O., Mbati, P. A., Robert, L. L. & Githure, J. I. 1994. Experimental infection of domestic goats with *Leishmania major* through bites of infected Phlebotomus duboscqi and needle inoculation of culture-derived promastigotes. *Vet. Res. Comm. 18:* 1562-1567.

Bray, R. S. 1982. The zoonotic potential of reservoirs of leishmaniasis in the Old World. *Ecology of Disease 1:* 257-267.

Heisch, R. B. 1957a. The isolation of *Leishmania* from a ground squirrel in Kenya. *East Afr. Med. J. 34:* 183.

Heisch, R. B., 1957b. The isolation of *Leishmania* from gerbils in Kenya. *J. Trop. Med. Hyg. 62:* 158-159.

Heisch, R. B. 1963. Is there an animal reservoir of kala-azar in Kenya? *East Afr. Med. J. 40:* 359-362.

Jahn, A. & Diesfeld, H. J. 1983. Evaluation of a visually read ELISA for serodiagnosis and sero-epidemiological studies of kala-azar in the Baringo District, Kenya. *Trans. Roy. Soc. Trop. Med. Hyg. 77:* 451-454.

Mutinga, M. J., Kaddu, J. B., Sang, D. & Arap Siongok, T. K. 1982. Studies of animal reservoirs of visceral leishmaniasis in Kenya. *East Afr. Med. J. 59:* 446-450.

Mutinga, M. J., Kihara, S. M., Lohding, A., Mutero, C. M., Ngatia, T. A. & Karanu, F. 1989. Leishmaniasis in Kenya: Description of leishmaniasis of a domestic goat from Transmara, Narok District, Kenya. *Trop. Med. Parasitol. 40:* 91-96.

Mutinga, M. J., Mutero, C., Ngindu, A. & Amimo, F. 1988. The isolation of leishmanial parasites from domestic goats and wild hosts in Kenya and the possible role of goats as reservoirs of Leishmaniasis. *Insect Sci. Applic. 9:* 347-349.

Mutinga, M. J., Ngoka, J. M., Schnur, L. F. & Chance, M. L. 1980. The isolation and identification of leishmanial parasites from domestic dogs in the Machakos District of Kenya, and the possible role of dogs as reservoirs of Kala-azar in East Africa. *Ann. Trop. Med. Parasitol. 74:* 139-144.

Ngoka, J. M. & Mutinga, M. J. 1977. The dog as a reservoir of visceral leishmaniasis in Kenya. *Trans. Roy. Soc. Trop. Med. Hyg. 71:* 447-448.

Ngoka, J. M. & Mutinga, M. J. 1978. Visceral leishmaniasis animal reservoirs in Kenya. *East Afr. Med. J. 55:* 333-336.

Ngumbi, P. M., Lawyer, P. G., Johnson, R. N., Kiilu, G. & Asiago, C. 1992. Identification of phlebotomine sand fly bloodmeals from Baringo Disrict, Kenya by direct enzyme-liked immunosorbent assay (ELISA). *Med. Vet. Entomol. 6:* 385-388.

Schaefer, K. U., Kurtzhals, A. L., Kager, P. A., Gachihi, G. S., Gramiccia, M., Kagai, J. M., Sherwood, J. A. & Muller, A. S. 1994. Studies on the prevalence of leishmanin skin test positivity in the Baringo District, Rift Valley, Kenya. *Am. J. Trop. Med. Hyg. 50:* 78-84.

Southgate, B. A. & Oriedo, B. V. E. 1962. Studies in the epidemiology of East African Leishmaniasis. I. The circumstantial epidemiology of kala-azar in the Kitui District of Kenya. *Trans. Roy. Soc. Trop. Med. Hyg. 56:* 30-47.

Van Der Lugt, J. J., Carlyon, J. F. & De Waal, D. T. 1992. Cutaneous leishmaniasis in a sheep. *J. S. Afr. Vet. Assoc. 63:* 74-77.

Voller, A., Bartlett, A & Bidwell, D. E. 1976. Enzyme immunoassays of parasitic diseases. *Trans. Roy. Soc. Trop. Med. Hyg. 70:* 98-106.

Wijers, D. J. B. & Mwangi, S. 1966. Studies on the vector of Kala-azar in Kenya. VI. Environmental epidemiology in Meru District. *Ann. Trop. Med. Parasitol. 60:* 373-391.

Williams, A. O., Mutinga, J. & Rodgers, M. 1991. Leishmaniasis in a domestic goat in Kenya. *Mol. Cell. Probes 5:* 319-325.