SEROSURVEY OF LEPTOSPIROSIS IN RODENTS, DOGS AND CATTLE IN SELECTED AREAS OF TANZANIA

MACHANG'U, R. S., MGODE G. and MPANDUJI D. G., Sokoine University of Agriculture, Faculty of Veterinary Medicine (Rodent Research), P. O. Box 3110, Morogoro, Tanzania

SUMMARY

Sera from 460 rodents, 207 dogs, and 374 cattle collected from different regions of Tanzania were examined by the microagglutination (MAT) assay for antibodies to *Leptospira icterohaemorrhagiae, L. canicola* and *L. hardjo*. Overall, 2.1% of rodent sera were positive at titres of 1:160 or higher to *L. icterohaemorrhagiae*. Thirty eight and 0.5% dog sera were reactive to *L. icterohaemorrhagiae* and *L.canicola* respectively, and 5.6% of bovine sera were reactive to *L. hardjo*. These results show that rodents and domestic animals are probably important carriers of leptospiras. To our knowledge, this is the first documented study of leptospirosis in Tanzania.

INTRODUCTION

Leptospirosis, which affects mammals and reptiles (Thierman, 1984) is a zoonotic disease of a world wide distribution (WHO,1967).In tropical Africa, this disease is prevalent but only limited studies have been documented (Njenga and Ouma,1992). 1992).

Leptospira interrogans, recognized for a long time as the pathogenic species of the genus *Leptospira*, consists of about 20 serotypes, with 180 serovariants (Faine,1982). Although it is generally accepted that *L. interrogans* has a broad host range, certain serovars e.g. *L. hardjo*, and *L. canicola* show some degree of host preference for cattle and dogs respectively, whereas *L. pomona* is frequently isolated from pigs (Thierman, 1984). Rodents can be infected by any of the diverse serovars without developing clinical symptoms (Faine,1982). The other species of the genus leptospira, *L. biflexa*, is non pathogenic.

In Tanzania, leptospirosis has been suspected in a number of pathological and clinical conditions (Semuguruka, 1974; Mtambo; Sinare, pers. communication). These unconfirmed reports have raised interest to research on the prevalence of this zoonotic disease. This paper presents the first study of seroprevalence of leptospirosis in rodents (rats), dogs and cattle in selected areas of Tanzania.

MATERIALS AND METHODS.

Antigens:

Stock antigens consisted of live cultures of *L. interrogans* serovars: *icterohaemorrhagiae*, *canicola*,and *hardjo* grown in Fletcher medium containing 10% (vol/vol) letospira enrichment medium (Difco Laboratories, Detroit, USA). The cultures were examined for the presence of contaminants under a dark-field (DF) microscope. Lightly contaminated cultures were diluted with phosphate buffered saline (PBS, pH 7.2) at a ratio of 1:9 culture:PBS and passed through sterile 0.2 μm,-cellulose microfilters, (Whatman Ltd, Maidstone, England) in re-usable microfilter holder units (Nalge, Rochester, USA). All antigens were kindly provided by Dr. W. Terpstra, of the WHO Collaborating Centre for Research on Leptospira and Leptospirosis, Royal Tropical Institute of Hygene, (KIT) Amsterdam, Netherlands.

The reference strains, RGA (*L. icterohaemorrhagiae*) and Hond Uttrecht (*L. canicola*), were used for the serology of rodents, and dogs respectively, and strain Hardjoprajitno (*L. hardjo*), was used for the screening of bovine sera. These antigens had undergone a series of subculturing in our laboratory over a period of 2 years.

Antigen standardization

One ml of contaminant free culture was transferred into 9 ml Ellinghausen-MacCullough-Johnson Harris (EMJH) leptospira medium (Difco Laboratories, Detroit, USA) in 15 ml srcew capped test tubes and incubated in a water bath with shaker (30°C,150 rpm) (GFL 1092, Burgwedel,Germany). Leptospiral growth was demonstrated by turbidity of the culture medium after 4 days of incubation, and was confirmed by DF microscopy. The density of leptospiras was standardized with PBS to $3x10^8$ leptospiras/ml on the MacFarland scale before use in the serological assay.

Serum collection

Blood sampling for sera was carried out in different areas of Tanzania (Tabs 1-3). Rodent blood (460 samples) was collected from the optical sinus of trapped *Mastomys natalensis* (field rats) and *Rattus rattus* using glass capillary tubes. Dog (207) and cattle (374) blood samples were collected in plain vacutainer[a] tubes (Becton and Dickinson,Meylan, France) by the saphenum, respectively jugular routes. The breed, or service of the animals studied were not taken into consideration during sampling. The blood samples were left for 1 hr at room temperature (RT) to clot, and then for another 1 hr at 4°C to allow clot retraction. Sera were separated into microcentrifuge tubes using sterile pasteur pipettes and stored at -20°C until used. Where necessary, low speed centrifugation (800 rpm) was done to enhance serum separation.

Control sera

Positive control sera were kindly supplied by KIT, Amsterdam, and DIFCO Laboratories (Detroit, USA). These sera had titres of 1:640, for *L. icterohaemorrhagiae*, 1:2,560 for *L. canicola*, and 1:5,120 for *L. hardjo*. For negative control, a 2-fold dilution of the stock leptospira antigen in PBS was used according to recommendations by KIT.

Serology

Microagglutination test (MAT) was performed in U-bottom microtitre plates (Nunc, Kamstrup, Denmark), as described by Cole et al., (1973) with minor modifications to accommodate simultaneous screening of 22 serum samples, and 2 controls per plate. Briefly, serial doubling dilutions of the test sera in PBS and the control sera were performed in the microtitre wells such that each well contained 50 μl vols. Aliquots of 50 μl of the reference leptospira serovar were then added to all 96 wells such that the final dilutions of the sera ranged from 1:20 to 1:160. The plates were tapped gently to homogenize the antigen-serum mixture and then incubated for 2 hr at 30°C.

The reactions were read under DF microscope (x 10 objective, x 10 ocular) and agglutination was recorded subjectively as 4+, or 100% (where all leptospiras appeared agglutinated) and 3+ and 2+ for 75% and 50% agglutination respectively. Conventionally, agglutination of below 50% was considered insignificant.

Establishment of agglutination titre

All sera that were positive at the preliminary screening i.e, with agglutination $\geq$ 2+ at dilutions of 1:160, were reexamined as above, at higher dilutions (up to 1:20,480) in order to establish the cut-off titre.

RESULTS

Antibody titres of sera (seropositivity) in rodents, dogs, and cattle against serovars *L. icterohaemorrhagiae*, *L. canicola* and *L. hardjo* are shown in Tables 1-3.

Seropositivity to *L. icterohaemorrhagiae* in rodent sera per area of study, in brackets) was: 4.6% (Morogoro-Sangasanga), 12.5% (Morogoro-Mtibwa) and 7.4% (Lushoto). No antibodies to leptospiras were detected in rodent sera from the other localities (Table 1). In dogs seropositivity to *L. icterohaemorrhagiae* was 58.9% (Lower Moshi) 34.6% (Morogoro urban), and 4% (Dar Es Salaam) For cattle, seropositivity was broadly spread across the studied areas, except Moshi Highlands (Tab. 2).

Table 1: Seropositivity of rodent sera to *Leptospira interrogans* serovar *icterohaemorrhagiae*

Area	N	+VE	%+VE
Mor-Sanga	65	3	4.6
Mor-Mtibwa	40	5	12.5
Lushoto	27	2	7.4
Mbinga	25	0	0
Singida	30	0	0
L.Rukwa L.Tanganyika basins	273	0	0
Total	460	10	2.1

DISCUSSION

This survey has shown that leptospirosis due to *L. icteroaemorrhagiae*, *L. canicola and L.hardjo* is prevalent in rodents, dogs, and cattle in the Morogoro, Dar Es Salaam, Lower Moshi and Lushoto areas of Tanzania (Tables 1-3).

Table: 2. Seropositivity of dog sera to *Leptospira interrogans* serovars *icterohaemorrhagiae* and *(canicola)*

Area	N	+VE	%+VE
Morogoro urban	78	27 (0)	34.6 (0)
Dar es Salaam	72	29 (1)	4 (1.36)
Lower Moshi	39	23 (0)	58.9 (0)
Singida	18	0 (NI)	0 (NI)
Total	190	79	38.16

(NI) Not investigated

Table: 3 Seropositivity of cattle sera to *Leptospira interrogans* serovar *hardjo:*

Area	N	+VE	%+VE
Lower Moshi	77	14	18
Moshi-highlands	37	0	0
Dar es-Salaam	17	2	11.7
Mbeya (Usangu valley)	168	2	1.19
Mwa-nza	75	3	4
Total	374	21	5.6

The locations where most of the seropositive animals originated lied in marshy lowlands or close to irrigated plantations. This was not unexpected, because leptospiras do survive for long periods of time if dischaged by vectors or carrier animals into warm and swampy environments (Faine, 1982).

The relatively high seroprevalence of *L. icterohaemorrhagiae* (58%) in dogs in lower Moshi appears to reflect a higher incidence of infection by leptospiras in this area, possibly due to extensive irrigation activities (sugar cane, rice farming). It is, however, striking that low seropositivity (0.5%) to *L. canicola*, which is canine adapted, was recorded with the dog sera. This suggests that this serovar is rare in the Lower Moshi localities.

Canine leptospirosis has often been associated with hunting dogs because of the swampy environments in which they stalk their hunt (Faine, 1982). For the case of rural Tanzania where most dogs are free "wanderers", infections in these animals would be expectedly high. Indeed most of the sera collected from the lower Moshi area came from vagabond dogs. Also, for the densely populated low lying urban centres, such as Morogoro, with relatively high rainfall and poor drainage systems, leptospirosis might be relatively high even in dogs that are kept restrained at most times.

The seropositivity of cattle to *L. hardjo*, suggests a wide distribution of the leptospira in these animals across Tanzania, indifferent of the livestock keeping system.

The presence of antibodies to in rats in the studied arcas indicate that they may be the source of infection for domestic animals. Rodents are the natural reservoirs of leptospiras, and studies elsewhere have demonstrated their importance in the maintenance of leptospiras in the environment, and their transmission of disease to susceptible livestock and humans (Lins and Lopez,1984; Waitkins, 1985; Everard, 1992). Additional studies are therefore required to establish the epidemiological patterns of leptospirosis in the studied areas, and in other areas of Tanzania. In addition to the role of rodents in the transmission of leptospirosis, wildlife-livestock interactions in areas neighbouring game reserves, and the seasonal migration of cattle in pastoral areas could present major contributing factors for a wider prevalence of leptospirosis across Tanzania.

REFERENCES

Cole, J.R., Sulzer, R.C. and Pursell, A.R. (1973). Improved microtechnique for the leptospiral microscopic agglutination test. J. Appl. Microbiol. Vol. 209:976-980.

Everard, C.O.R. 1992. The epidemiology of leptospirosis. In Proceedings of a CEC/STD 3 Research Meeting "Leptospirosis on African Continent" Harare Zimbabwe.

Faine, S . (1982). Guidelines for the control of leptospirosis, WHO Publ. Offset. No. 67.

Lins, Z.C. and Lopez, M.L. (1984). Isolation of leptospira from wild forest animals in Amazonian Brazil. Transact. Roy. Soc. Trop. Med. Hyg. 78:124-126.

Machang'u, R.S. (1992). Leptospirosis in tropical and subtropical Africa. In Proc. CEC/STD 3 Res. Meeting "Leptospirosis on African Continent, Harare Zimbabwe.

Semuguruka, W.(1974). Suspected case of leptospirosis in tbe dog. Annual Central Veterinary Report, DSM.

Thierman, A.B. (1984). Leptospirosis current Development and Trends J. Am. Vet. Med. Assoc. 6:722-725.

Waitkins, S.A., Wanyangu, S. and Palmer, M. (1985). The copy as a rodent reservoir of leptospira infection in Great Britain. J. Hyg. Camb. 95:409-417.

WHO (1967) Current problems in leptospirosis research. Wld. Hlth. Org. Techn. Rep. Ser., No 380.