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LEPTOSPIROSIS IN ANIMALS AND HUMANS IN SELECTED AREAS OF TANZANIA

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Abstract. A serosurvey was carried out in selected areas of Tanzania to determine the prevalence of leptospirosis in animals and humans. Sera of rodents (n=537), cattle (n=374), dogs (n=208) and humans (n=375) were screened for antibodies by microagglutination (MAT) procedure. The areas studied included swampy or irrigated sugar cane and rice fields (Mtibwa-Morogoro, Sangasanga-Morogoro, Lower Moshi), highlands (Moshi Highlands, Lushoto, Mbizi Forests-Rukwa), pastoral plateaus (Singida, Mwanza, Mbeya, Mbinga), and a Lake basin (Lake Rukwa). *Leptospira interrogans* serovars *icterohaemorrhagiae, hardjo, canicola, pyrogenes* and *grippotyphosa* served as reference antigens in the MAT assay. Antibodies to serovar *icterohaemorrhagiae* were demonstrated in 1.9% of the sera of examined rodents (*Mastomys natalensis*, *Rattus rattus*). Cattle sera showed the presence of antibodies to serovars *hardjo* (5.6%) and *pyrogenes* (1.9%) respectively, and dog sera showed antibodies to serovars *icterohaemorrhagiae* (37%), and *canicola* (0.5%) respectively. A single sample of the human sera agglutinated with serovar *grippotyphosa.* In an attempt to isolate leptospires from urine of 1021 cattle at a slaughterhouse in Morogoro, 7 isolates were obtained. This study has shown that leptospirosis is a potential public health hazard in certain areas of Tanzania.

Key words: Leptospirosis, Seroprevalence, Microagglutination, Isolation.

INTRODUCTION

Leptospirosis is a bacterial disease of worldwide distribution, which affects all mammals, including humans, livestock and wildlife (WHO,1967; THIERMAN, 1984). In humans, leptospirosis may present as a hyper-acute disease leading to quick death, but can also show as a mild febrile disease – «pyrexia of uknown origin» (PUO) (TERPSTRA, 1992). In both cases, it is difficult to diagnose leptospirosis clinically, and unless the clinician specifically looks for it, leptospirosis can be easily overlooked or misdiagnosed.

In theory, any mammal can be infected with one or more serovars of *L. interrogans* but certain serovars show some degree of host specificty. Examples of such serovars and their preferential host (in brackets) include serovars *icterohaemorrhagiae* (rodents), *canicola* (dogs), and *hardjo* (cattle) (THIERMAN, 1984; EVERARD, 1992).

Rodents are no doubt the natural reservoir hosts of many leptospiral serovars. In the tropics, peridomestic and field rodents, such as *Rattus rattus* (L., 1758), *Mastomys natalensis* (Smith, 1834), *Cricetomys gambianus* (Waterhouse, 1840) and *Arvicanthis niloticus* (Desmarest, 1822) are known to be the primary reservoirs of leptospires. Terri-

tory marking canivores are probably the second most highly infected animals, and intra and interspecific transmission within infected hosts may persist in an area for a long time, especially if climatic conditions are favourable for the intermittent survival of the leptospires in the environment (EVERARD, 1992).

Leptospirosis has been known for about a century, since WEIL (1886) and INADA (1916) described this disease and its etiological agent for the first time. However, knowledge about the infectious agent and the prevalence of this disease in many tropical countries is limited. Consequently, the epidemiology of this disease in livestock and humans and its impact on public health in these parts of the world is poorly documented and its assessment largely based on speculations (ELLIS, 1984).

In the East African region, animal leptospirosis was first documented in Kenya and Uganda by BALL (1966). Additional reports on leptospirosis from this region were by KRANENDONK *et al.* (1968) who isolated three new serovars (*kanana*, *lambwe* and *njenga*) from the Coastal Province of Kenya. Human infection, also in Kenya, was first reported by FORRESTER *et al.* (1969), and by DE GEUS *et al.* (1977). These reports were complemented by the isolation of the infectious agent and the description of a new serovar (*kibos*) (DIKKEN *et al.*, 1981; KMETY & DIKKEN, 1993).

Serodiagnosis is the most common approach used to survey leptospirosis in environments where the disease has not been previously reported. Over the years, the microagglutination-test (MAT) has been established as the standard assay for the diagnosis of leptospirosis (COLE *et al.*, 1973; KORVER, 1992; FERESU *et al.*, 1995). The MAT is, however, laborious and time consuming and requires maintenance of numerous live reference antigens. It may also fail to detect antibodies to unique serovars in a newly studied area, where reference strains have to be used instead of indigeneous strains. On the other hand, the advantage of MAT is that it can be carried out even in modestly furnished laboratories because it does not require expensive equipment. For the clinician, definitive diagnosis of leptospirosis is the isolation of the infectious microorganism from the host, but isolation is difficult, a lenghthy process, and often unsuccessful even in specialized laboratories (TERPSTRA, 1992).

This paper reports on a study of seroprevalence of leptospirosis in rodents, cattle, dogs, and humans, carried out in selected areas of Tanzania, and on the isolation of leptospires from urine of cattle submitted for slaughter at the Morogoro town slaughterhouse.

MATERIAL AND METHODS

Leptospira reference cultures (antigens)

Reference strains for use in MAT were kindly supplied by the WHO Reference Laboratory at the Royal Tropical Institute of Hygiene (KIT), Amsterdam, Netherlands. The reference strains were maintained at room temperature (20°-27°C) in Fletchers's medium (DIFCO, Laboratories, Detroit, USA) supplemented with leptospira enrichment medium (DIFCO), and 5-fluorouracil (FU) (0.5%). Growth and purity of the cultures was monitored by dark field (DF) microscopy at seven day intervals, and subculturing in fresh

medium was done either when the cultures became too dense, or after 28 days of growth. Contaminated cultures were purified by either subculturing in Ellinghausen-McCullough's medium, as modified by Johnson and Harris (EMJH), supplemented with cyclohexamide (0.5 mg/ml) as selective inhibitor, or by suspending the cultures in EMJH and then filtering into fresh Fletcher's medium through syringe adaptable 0.22m filters (Millipore Corporation, Bedford Ma. USA).

Collection of test sera

Rodents (n=537) were trapped from swampy or irrigated agricultural areas with sugar cane, and rice farms (Mtibwa-Morogoro, Sangasanga-Morogoro, and Lower Moshi); from highland valleys and tropical forests (Lushoto, and Mbizi Forest-Rukwa respectively), and from the Singida Plateau (Fig. 1). Retroorbital blood was collected from these rodents and the sera were separated and stored at -20° C until tested. All rodent sera were tested against serovar *icterohaemorrhagiae*.

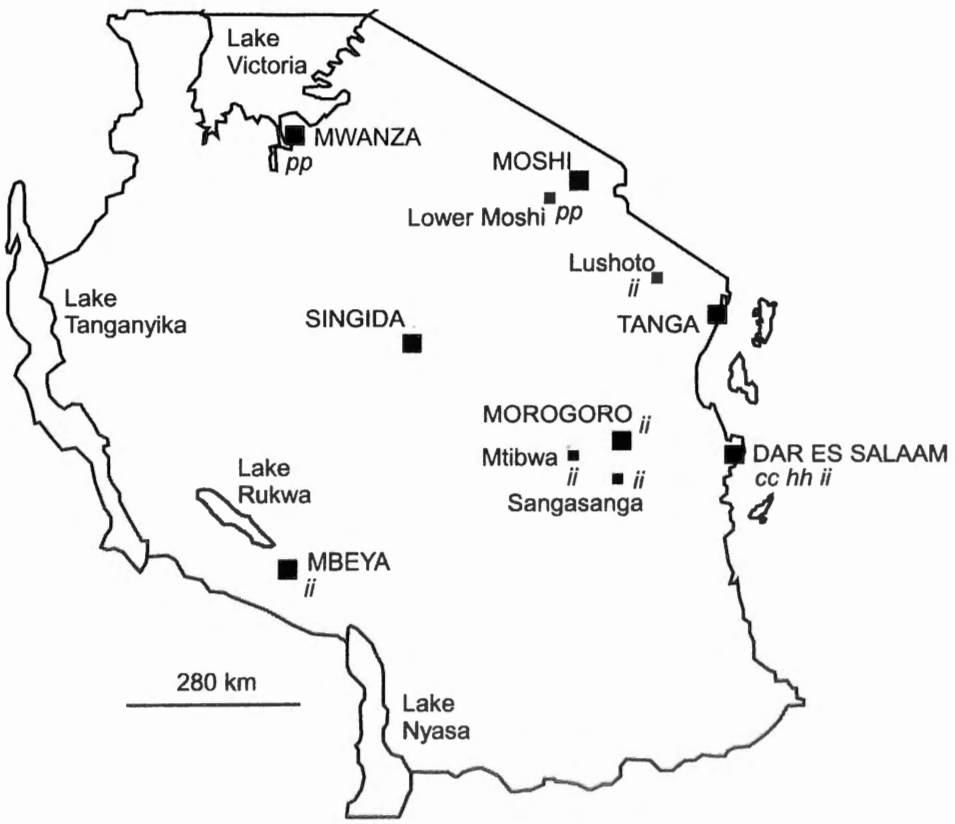

Fig. 1. – Map of Tanzania showing areas of seroprevalence for leptospirosis. cc=canicola; hh=hardjo; ii=icterohaemorrhagiae; pp=pyrogenes.

Cattle were sampled by taking jugular blood of 374 animals originating from dairy farms and ranches in Lower Moshi and Moshi highlands respectively, and from pastoral plains (Iringa, Mbeya, Mbinga-Ruvuma, and Mwanza). Samples were also taken in the Dar es Salaam urban area (Fig. 1). All cattle sera were tested against serovar *hardjo*, and then 360 of these samples were tested against serovar *pyrogenes*.

Dogs (n=208) were sampled by venipuncture of the saphenum veins. Of these, 39 were domestic, unrestrained dogs from different villages in the Lower Moshi area, and 18 were from villages in the Singida plateau. The rest of the dogs had been brought in for dipping at various veterinary clinics in Dar es Salaam town (73 dogs), and at the Sokoine University Veterinary Clinic, Morogoro (70 dogs). The sera were first tested against serovar *canicola*, and in a second run, the sera from Singida were excluded and the remaining were tested against serovar *icterohaemorrhagiae*.

Human sera were donated by 159 young adult male cane cutters from Kilombero Sugar Company (Fig. 1) and another 216 sera were kindly provided by Dr. J. Shao of the Department of Microbiology, Muhimbili University College of Health Sciences (MUCHS), Dar es Salaam. These sera were screened against serovars *icterohaemorrhagiae* and *grippotyphosa*. None of the domestic animals and humans showed any clinical signs suggesting leptospirosis.

Antibody detection

All sera were screened for antibodies against leptospires using MAT as described by COLE *et al.* (1973). Briefly, the sera (100µl amounts) were titrated with phosphate buffered saline (PBS) in `U' microtitration plates to obtain an initial titre range of 1:20-1:160. Equal volumes of antigens grown in liquid EMJH medium to a density of approximately 3×10^8 leptospires/ml on the MacFarland scale were then added and the plates incubated at room temperature for 2 h. The reactions were then examined for agglutination by DF microsocopy. A serum was considered positive if 50% or more of the microorganisms in the microtiter well agglutinated at titre 1:160. These positive sera were subsequently titrated to 1:20480 in PBS and tested again with the antigen to establish the antibody titre. Control positive and negative sera were supplied by DIFCO Laboratories, Detroit, and KIT, Amsterdam, Netherlands, respectively.

Isolation of leptospires

Urine samples were collected from freshly excised bladders of 1021 clinically healthy cattle at the Morogoro abbatoir. Urine samples (20 µl) were examined for spirochetes by DF microscopy, and quantities of 5 ml were inoculated in McCartney bottles containing 15 ml Fletcher's medium with 10% leptospira enrichment. These cultures were then incubated (30° C) with shaking for 28 days and examined for leptospira growth at 7 day intervals.

RESULTS

The seropositivity to different leptospira serovars per species and locality are shown in Table 1.

TABLE 1

Seropositivity for leptospirosis in animals and humans in Tanzania

Sampled group	*N positive/N tested*	*% Positive*	*Serovar*	*Locality*
Rodents	10/537	1.8	*icterohaemorrhagiae*	Sangasanga, Mtibwa, Lushoto
Cattle	21/374	5.6	*hardjo*	Lower Moshi, Dar es Salaam, Mbeya
Cattle	7/360	1.9	*pyrogenes*	Mwanza, Mbeya
Dogs	79/208	38	*icterohaemorrhagiae*	Lower Moshi, Morogoro
Dogs	1/208	0.5	*canicola*	Dar es Salaam
Humans	1/375	0.3	*grippotyphosa*	MUCHS – Dar es Salaam

Seroprevalence in rodents

Seropositivity to serovar *icterohaemorrhagiae* was recorded with 10 out of 537 (1.9%) rodent sera examined. The positive sera, (number in parentheses) originated from Mtibwa-Morogoro (5), Sangasanga-Morogoro (3) and Lushoto (2) (Fig. 1). Of these sera, 7 were from *Mastomys natalensis,* and 3 were from *Rattus rattus*.

Seroprevalence in cattle

Out of 374 cattle sera screened, 21 (5.6%) agglutinated against serovar *hardjo,* and 7 (1.9)% agglutinated against serovar *pyrogenes* (Tab. 1). Fourteen of the *hardjo*-positive sera were from Lower Moshi, 2 from Dar es Salaam town, 2 from Mbeya, and 3 from Mwanza. Three of the *pyrogenes*-positive sera were from Mwanza and 4 from Mbeya (Fig. 1).

Seroprevalence in dogs

Out of 208 dog sera screened against serovar *icterohaemorrhagiae,* 79 samples (38%) reacted positively. These were from Lower Moshi (23 samples), Dar es Salaam town (29 samples), and Morogoro town (27 samples). Only one out of 208 dog serum samples (0.5%) agglutinated against serovar *canicola*. This serum originated from Dar-es-Salaam town (Fig. 1).

Seroprevalence in humans.

Out of the 375 human serum samples, one (0.3%) reacted positively to serovar *grippotyphosa*. This sample was obtained from MUCHS, Dar es Salaam. None of the human sera reacted against serovar *icterohaemorrhagiae*.

Isolation of leptospires from cattle urine.

Out of 1021 cattle urine samples screened, 7 were positive for leptospires. The microorganisms were isolated and purified in Fletcher's and EMJH media, for subsequent characterization.

DISCUSSION

This is the first report on leptospirosis in Tanzania. Although the study focused on relatively few areas, it has provided substantial evidence of leptospiral prevalence in rodents, cattle, dogs and humans in Tanzania. The prevalence of serovar *icterohaemorrhagiae* in rodents was demonstrated with two species (*M. natalensis* and *R. rattus*) in the Morogoro area. These two species are the most abundant small mammals in Tanzania, and could therefore be the most important rodent reservoirs of leptospires in Tanzania. At least *M. natalensis* has been shown to be a potential carrier of other serious diseases in Tanzania (LEIRS *et al.*, 1988; KILONZO *et al.*, 1992).

The overall highest seroprevalence (38%) was demonstrated with serovar *icterohaemorrhagiae* in dogs in the Lower Moshi area; however, this serovar was not prevalent in the dogs from the other areas studied. Dogs may, therefore, be important maintenance hosts of serovar *icterohaemorrhagiae* in the Lower Moshi area. It is in agreement with previous reports that canines are, next to rodents, the most common carriers of leptospires, and that the dog is a potential transmitter of leptospirosis to humans in the domestic environment (EVERARD, 1992). Seroprevalence in humans in the Lower Moshi area was not studied.

The observed high seroprevalence of leptospirosis in dogs in Lower Moshi could be due to intraspecific transmission among dogs because in this area dog keeping is common; at the same time, dogs are never restrained and therefore are able to stray in the neighbourhood.

The seroprevalence of serovar *hardjo* in cattle is in agreement with previous reports that this serovar is frequently found in bovines (FERESU, 1987). In this study, serovar *hardjo* appeared uncommon in cattle in the pastoral plain (Mwanza), where serovar *pyrogenes* was the prevalent one. Apparently certain serovars might be common in some areas of Tanzania but not in others.

Seropositivity in humans was demonstrated with only one serum sample against serovar *grippotyphosa*. This prevalence can be considered an underestimate because of the limited number of sera and the serovars tested. Additional studies on humans are desirable, particularly in the Lower Moshi area, to see whether there is a correlation between the high seroprevalence recorded with dogs, and human infection.

The isolation of 7 leptospires from 1021 cattle urine complemented the serological findings. However, this isolation (0.7%) may not be a true reflection of the number of cattle infected because the rate of success in isolation of leptospires from urine is relatively low (FAINE, 1982). Additional studies involving isolations from kidneys could give a clearer prevalence rate in the cattle.

The seroprevalence and the isolation of leptospires demonstrated in this study came as no surprise to us because the environment in many parts of Tanzania is favourable for the survival of leptospires. Such environment includes the sugar cane and rice plantations, which are in marshy or irrigated lands; and flood basins, where fishing and livestock grazing are commonly practiced. Also, many natural reservoirs and maintenance hosts of leptospires are found in all these areas. The existence of reports on the prevalence of leptospirosis in livestock and humans in the neighbouring countries of Kenya, Uganda, and Zimbabwe further supports our findings for Tanzania.

Leptospirosis is a health hazard that has been overlooked in Tanzania and elsewhere in Africa (ELLIS, 1984; MACHANG'U 1992). There is a need to alert all occupational groups at risk of the dangers of infection. Public health professionals, veterinarians, and physicians should consider including leptospirosis in the diagnoses of all clinical PUO cases of animals and humans, and in other conditions with symptoms suggesting leptospirosis. By doing so, the extent of leptospirosis and its economic importance will be known and preventive approaches could be initiated.

Future studies will aim at further isolation and characterization of endemic serovars, and at establishing the epidemiological patterns of leptospirosis in different parts of Tanzania.

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