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DOMESTIC RODENTS AS RESERVOIRS OF PATHOGENIC *LEPTOSPIRA* ON TWO CITY OF HARARE FARMS: PRELIMINARY RESULTS OF BACTERIOLOGICAL AND SEROLOGICAL STUDIES

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Abstract. Bacteriological and serological studies were carried out to determine the role domestic rodents play in transmitting leptospirosis on two City of Harare farms. Rodents were trapped and their kidneys and urine cultured for *Leptospira*. The rodents and volunteer blood donors, from farm workers and their families, were bled and the sera screened for antibodies against representative strains of eight serogroups of *Leptospira* using the Microscopic Agglutination Test.

Rattus rattus was the most abundant rodent caught and yielded the majority of the *Leptospira* isolates. The prevalence of leptospiral titres at a serum dilution of 1:100, was 62.5% for the rodents and 82% for the volunteer blood donors. The most common titres in both the rodents and humans were to antigens from the Icterohaemorrhagiae, Pyrogenes and Grippotyphosa serogroups. The results suggest that leptospirosis is a common occupational disease of workers on the two farms which is transmitted to them by rodents.

Key words: rodents, reservoirs, *Leptospira*.

INTRODUCTION

Leptospirosis is an important zoonosis which affects all mammals and can be transmitted among domestic, wild animals and man. The clinical picture in man may vary considerably, from mild illness resembling flue, to severe illness as in Weil's disease where there is renal failure and meningitis. Death from leptospirosis may occur in 5-30% of untreated human cases (WAITKINS, 1983).

Rodents living in close association with humans have been found to be the most important reservoirs of leptospires (FAINE, 1982). They then transmit the leptospires to man either directly or indirectly through an intermediary domestic animal species. Reservoir rodents maintain the organism in their kidneys and pass it to humans by excreting urine in homes, paddocks, water-ponds, sewers and on farms where humans work or live. Therefore although leptospirosis may not be ranked as one of the most important dis-

eases universally, it can be very common in certain occupational groups such as agricultural workers, refuse collectors, sewer and sewage workers who are exposed to environments frequented by these rodents (WAITKINS, 1983). The principal ports of entry of the leptospires are: the skin especially when it has been wounded, abraded or softened by continued contact with water; and the mucous membranes of the eyes, nose and throat (FAINE, 1982).

Leptospirosis is known to be more prevalent in tropical and subtropical regions, particularly in the communal areas and on farms, due to the low standard of living, labour intensive agricultural practices, limited sanitation facilities and poor animal handling techniques (FERESU, 1990). Most people living in these communities are usually bare-foot and may be exposed to leptospires from the rodents in pastures, ponds and rivers. Their children often play and swim in areas frequented by rodents.

Research on leptospirosis has been very limited on the African continent, but the few studies done have demonstrated that it exists in all the countries where studies have been undertaken (DIALLO & DENNIS, 1982). In Zimbabwe, although evidence of leptospirosis was established as early as 1956, there have been very few documented reports, most of which were entirely based on clinical diagnosis (MOSSOP, 1974; WILES, 1979). There have also been limited serological surveys in humans (GRAF, 1965), in dogs (BANKS & PIGOTT, 1979) and two large surveys in cattle (SWANEPOEL *et al.*, 1975; FERESU, 1987). A few isolation studies have resulted in the discovery of five new serovars of *Leptospira*: zimbabwe; mombe; mhou; marondera and proposed ngavi (FERESU *et al.*, 1993; 1994; 1996; FERESU, unpublished results).

The purpose of the current study was to investigate the role of rodents in transmitting leptospirosis to humans on two City of Harare farms using both cultural and serological tests, since a strain of *Leptospira* had previously been isolated from a rat on one of the farms (Central Veterinary Research Laboratory Reports, 1978).

MATERIAL AND METHODS

The study areas

The Harare Municipality owns several farms at the periphery of the City. It uses these farms to purify sewage effluent whose quality cannot be discharged directly into the river system, by flood irrigating the pastures and allowing the water to be filtered naturally by the ground. The green pastures are used for cattle ranching.

The two farms are situated 30 km south west of the University of Zimbabwe. At the time of the study, Farm A had 67 while farm B had 41 permanent workers. The workers were allowed to stay with their families, bringing the total populations of the farms to 350 and 250 respectively. Each household was provided with a decent brick main house and was allowed to construct a pole and dagga hut to use as a kitchen. The quality of these kitchens was not controlled, making them very varied, with some being quite poor and allowing for heavy infestation with rodents.

Each household was allocated a piece of land for gardening which was also flood irrigated with sewage effluent. Thus the poor kitchens and potential extensive exposure of the farm workers and their families to flood irrigated pastures and gardens, presented with conditions conducive to the transmission of leptospirosis.

Trapping of rodents

The rodents of interest were *Rattus rattus* (L., 1758), *Mastomys* sp., *Mus musculus* L., 1758, *Rhabdomys pumilio* (Sparrmann, 1784), *Otomys angoniensis* Wroughton, 1906 and *Aethomys chrysophilus* (de Winton, 1897). They were trapped in and around human dwellings, in gardens and in storage sheds on the two farms over a period of approximately two years (January 1995 to October 1996), encompassing two wet and two dry seasons. The rodents were identified using morphological characters such as size and shape of body, colour of fur, length of the head in relation to the tail, size of the rear feet, relative size of the ears and eyes, and shape of droppings (GWINNER *et al.*, 1990). The identifications were verified by the National Museums of Zimbabwe.

Each rodent species was further grouped according to sex and age. Only two age groups were considered, the juveniles and the old, the latter having live weights (grammes) in excess of: 10, *M. musculus;* 20, *R. pumilio;* 25, *Mastomys* sp.; 50, *A. chrysophilus*; and 120, for both *R. rattus* and *O. angoniensis* (GWINNER *et al.*, 1990).

Isolation studies

Urine from the trapped rodents was collected directly by sterile bladder tapping or by flushing the bladder with 1 ml of 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS), pH 7.2. Whole kidneys were aseptically placed in a sterile plastic stomacher bag containing 10 ml of 1% BSA in PBS and homogenized. The urine and kidney homogenates were then serially diluted (10^{-1} through 10^{-4}) in 1% BSA in PBS. From each dilution, 1 ml was inoculated into a tube containing 9 ml of semi-solid modified Ellinghausen and McCullough (EMJH) medium (JOHNSON & HARRISON, 1967) with 5-fluorouracil (100 mg/ml) added. The tubes were incubated at 29°C for 12 weeks and checked weekly for characteristic leptospiral growth by darkfield microscopy.

Bleeding

Rodents. Blood from the trapped rodents was collected directly from the abdominal aorta. The sera were stored at -20°C until tested.

Farm workers. Volunteer blood donors were solicited among the farm workers and members of their families. Each volunteer was bled four times (at the beginning of each season) to determine their serological status during the course of the study. The sera were stored at -20°C until tested.

Questionnaire. A questionnaire was administered to the donors at the time of the third blood collection. The questions asked related to sex, age, period of stay on the farm, occu-

pational duties, illness with leptospirosis related symptoms during the course of the study and the level of exposure to contaminated environments.

Serological studies

Both the rodent and human sera were screened for leptospiral antibodies using the microscopic agglutination test (COLE *et al.*, 1973) at 1: 100 dilution. The antigens used included 13 representative strains of eight *Leptospira* serogroups: Icterohaemorrhagiae (strains RGA & SBF 23); Pyrogenes (SBF 20); Grippotyphosa (SBF 32 & Moskva); Australis (SBF 3 & Jez brat); Mini (Sari); Ballum (Mus 127); Hebdomadis (SBF 5, SBF 40) and Tarassovi (SBF 16, Perepelitsin). All SBF strains are Zimbabwean strains which were included to enhance positivity as more and higher titre reactions are often observed when indigenous strains are used rather than exotic reference strains (FAINE, 1982).

A serum was considered positive when 50% or more of the leptospires agglutinated.

Malaria. The human blood was also examined for malaria parasites after staining with the Giemsa stain. This was done to exclude malaria as it presents with symptoms similar to those of leptospirosis.

RESULTS

Trapping

The rodent species trapped over the four seasons are presented in Table 1. *Rattus rattus* and *Mastomys* sp. were the most abundant species caught throughout the trapping period. A large number of *R. pumilio* were also caught with most of them being trapped during the first dry season. *Aethomys chrysophilus*, *O. angoniensis* and *M. musculus* were rare and seasonal.

TABLE 1

Species distribution and numbers of rodents trapped at the two City of Harare farms during the four seasons

Rodent species	*Seasons*				
	1st Wet	*1st Dry*	*2nd Wet*	*2nd Dry*	*Total*
R. rattus	55	99	92	47	293
Mastomys sp.	6	44	21	14	85
R. pumilio	4	32	0	8	44
M. musculus	0	0	3	0	3
A. chrysophilus	0	0	4	1	5
O. angoniensis	0	5	0	2	7
Total	*65*	*180*	*120*	*72*	*437*

Isolation studies

A total of 52 strains were isolated from the rodents with most (46) being obtained from *R. rattus* (Table 2). More isolates (41) were obtained during the second wet season than during the first wet (5) and dry seasons (each 3). Forty of these isolates were from kidneys while the remaining 12 were from urine. Thus 12 rodents had isolates from both kidneys and urine, making the overall isolation rate 9%.

TABLE 2

Number of leptospiral isolates from each rodent species, overall % sero-positivity and sero-positivity of the sera within each rodent species

Rodent species	*Number trapped*	*Number of isolates*	*Overall % sero-positivity*	*% sero-positivity within species*
R. rattus	293	46	49	73
Mastomys sp.	85	2	7	35
R. pumilio	44	0	6	57
M. musculus	3	4	0.5	67
A. chrysophilus	5	0	0	0
O. angoniensis	7	0	0	0
Total	*437*	*52*	*62.5*	-

- = non-applicable.

Serological studies

Rodents. A large number, 62.5%, of the rodents had titres against one or more of the 13 *Leptospira* antigens. High percentages of *R. rattus, Mastomys* sp., *R. pumilio* and *M. musculus* had titres to the leptospiral antigens (Table 2). All the *O. angoniensis* and *A. chrysophilus* trapped were sero-negative.

More adults than juveniles were sero-positive. Sero-positivity was, however, not dependant on sex or season. Most rodent sera were positive against representative antigens of serogroups Pyrogenes, Icterohaemorrhagiae and Grippotyphosa (Table 3).

Blood donors. A total of 182 volunteer blood donors was initially recruited (102 from Farm A and 80 from Farm B) but 27 dropped out during the course of the study. One hundred and fifty (82%) volunteers had positive titres against one or more *Leptospira* antigens for at least one of their four bleeds. There was no particular trend in sero-positivity of the blood donors over seasons, although the percentage sero-positivity remained quite high over the whole sampling period.

The reactions with the different antigens were similar to those observed for rodents, with the most common titres being against representative strains of serogroups Icterohaemorrhagiae, Pyrogenes and Grippotyphosa (Table 3). This implies possible trans-

mission of leptospirosis from rodents to the volunteer farm workers and members of their families.

TABLE 3

Comparison of % sero-positivity to the various Leptospira *serogroups between human and rodent sera*

Antigen	*% sero-positive*	
	Rodent sera	*Human sera*
Pyrogenes	40	66
Icterohaemorrhagiae	22	69
Grippotyphosa	17	43
Australis	12	15
Mini	10	16
Autumnalis	8.5	21
Javanica	8	10
Ballum	6	8
Hebdomadis	3	13
Tarassovi	2	12

Questionnaire responses. People who had stayed on the farm for over 10 years were more sero-positive (51%) than those who had stayed for shorter periods (43%). More family members who donated blood (64%) were sero-positive than the volunteer municipal employees (34%). Most of the sero-positive donors remembered having suffered from leptospirosis related illnesses such as fever (84%), chills (67%) and haemorrhages (38%) during the study period. From their responses, 99% of the sero-positive volunteers had been exposed to the effluent used to flood irrigate the pastures and gardens and in most cases (85%) contact had been made with the effluent without protective clothing.

Malaria. No malaria parasites were detected in any of the blood samples.

DISCUSSION

This survey is the first comprehensive study to determine the role domestic rodents play as maintenance hosts of *Leptospira* and to demonstrate leptospirosis as a possible occupational disease of farm workers in Zimbabwe.

The results indicate *R. rattus* as the most abundant and important carrier of *Leptospira* on two City of Harare farms. *Rattus rattus* is one of the ubiquitous rodent species known to maintain leptospires throughout the world, and as few as two shedder rats per hectare can maintain leptospirosis within a population and cause epidemics in farm workers (FAINE, 1982).

Although the isolates have not yet been identified, the serological studies indicate that, in Zimbabwe, domestic rodents may be important reservoirs and transmitters of strains of serogroups Pyrogenes, Icterohaemorrhagiae and Grippotyphosa to humans. Transmission of strains of similar serogroups by domestic rodents has been observed elsewhere on the African continent, with rats having been shown to be carriers of serovar icterohaemorrhagiae in Egypt (McGuire & Myers, 1957); Tunisia (Bakoss & Chadli, 1965) and South Africa (Herr *et al.*, 1982), while serovar grippotyphosa has been isolated from *Mus musculus* in Egypt (Barsoum *et al.*, 1973).

Our results can however only be viewed as being suggestive as other possible sources of leptospiral infection and transmission on the farms were not investigated. Strains of *Leptospira* in municipal raw sewage, have been known to survive sewage treatment (Jones *et al.*, 1981), thus the effluent used to irrigate the pastures and gardens may have initially contained strains of *Leptospira* from carrier convalescents in the municipal population. Transmission of *Leptospira* from cattle is also possible as strains of the three serogroups have been isolated from Zimbabwe beef cattle (Feresu, 1990).

The results indicate that leptospirosis is a common occupational disease of farm workers and members of their families as the majority of the blood donors had antibodies against *Leptospira* and recalled having suffered from leptospirosis related symptoms. In Zimbabwe leptospirosis is not commonly included in differential diagnosis as it presents with symptoms similar to the more well known parasitic, viral and other bacterial infections such as malaria, hepatitis, rickettsia, Rift Valley fever and brucellosis. No malarial parasites were demonstrated in the blood of our study subjects. Thus the results of this study suggest that leptospirosis should be included in differential diagnosis, particularly for those patients from high risk occupational groups.

The higher numbers of family members with antibodies to *Leptospira* antigens may be explained by the practice on the farms where the municipal employees are supplied with protective clothing during working hours while the family members usually work barefoot in the flood irrigated gardens, children enjoy playing in mud and women cook and spend more time in rodent infested kitchens. However, although the municipal employees used protective clothing, their level of sero-positivity was still high (34%), because the wearing of gloves maybe impracticable when performing some chores, cattle may splash the effluent during handling and the effluent can seep in boots making them more hazardous than protective (Faine, 1982).

The present study has once again demonstrated the presence of strains of the genus *Leptospira* on the African continent and the need to carry out further research to determine the prevalence and importance of leptospirosis on the continent.

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