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Leptospira Serovars Circulating Among Human, Cattle and Goats with Associated Risk Factors in Ngara and Kibondo Districts, North-Western Tanzania

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Abstract

Leptospirosis is a neglected zoonotic disease, prominent in north-western Tanzania where interactions among humans, livestock and wildlife is high. This study therefore, assessed *Leptospira* seroprevalence and associated risk factors by screening *Leptospira* serovars from blood sera of cattle, goats and humans in Ngara and Kibondo Districts using Microscopic Agglutination Test (MAT). Blood sera were positive for seven *Leptospira* serovars in which Sokoine, Sejroe and Grippotyhposa had higher seropositivity of ≥ 20.9%, whereas *Leptospira* serovars Kenya, Pomona, Lora, and Bataviae had seropositivity of < 10%. Variation of seropositivity and seroprevalence between Ngara and Kibondo Districts was insignificant ($p = 0.0718$) but varied significantly between humans, goats and cattle ($p = 0.0183$). *Leptospira* serovars Sejroe and Grippotyphosa were present in humans, goats and cattle. Sokoine, Pomona and Bataviae were co-positive in humans and goats, while serovars Kenya and Lora were co-positive in humans and cattle, indicating the possibility of dualistic transmission of *Leptospira* serovars in the ecosystem. Suggested risk factors associated with acquiring *Leptospira* bacteria were drinking contaminated water, feeding contaminated food and farming in contaminated soils. Prevalence of *Leptospira* in the current study alerts for health and economic risks in north-western Tanzania, which requires intensive education programs on leptospirosis transmission and avoidance.

Keywords: *Leptospira*, serovars, seropositivity, zoonosis, serum, Kibondo, Ngara, cattle, goat.

Introduction

Leptospirosis is a zoonotic, neglected contagious bacterial disease caused by bacteria of the genus *Leptospira* (Evangelista and Coburn 2010, Assenga et al. 2015). Soil is the primary natural reservoir for the bacteria, and its outbreak is mainly associated with heavy rainfall and floods that flush bacteria out to the soil. The disease affects humans, domesticated animals and feral animals mainly through contact or exposure to soil, water or vegetation laden with leptospires (Mgode et al. 2015). The bacteria can enter the human body or intermediate host through cuts, wounds, abrasions and mucous membranes of the throat, nose or eyes.

Different species of rodents, wildlife and domesticated animals are potential reservoirs for the pathogens that reside in their kidney tubules and get discharged through urine (Assenga et al. 2015, Msemwa et al. 2021). The pathogens in the urine excreted by an infected animal in soil, water or vegetation

may get into humans or any other animal exposed to or in contact with it. In this case, people engaged in fishing, livestock breeding, farming, cleaning sewers, and veterinary and abattoir workers risk contracting the disease (Garba et al. 2017, Mgode et al. 2021). People living in or contiguous to bushes or jungles and those carrying their activities in wetlands and water bodies are also at higher risk of contracting the disease (Mgode et al. 2019).

Leptospira pathogens exist in various forms (serovars), distinguished by their antigenic properties (Ahmad et al. 2005). Worldwide there are about 250 *Leptospira* serovars, whereas, in Tanzania, about ten serovars are known. Six serovars, namely Sokoine (Icterohaemorrhegia), Serjoe, Hebdomadis, Grippotyphosa, Ballum and Australis, were shared among humans, livestock and wild animals ungulates in Katavi and Rukwa regions (Assenga et al. 2015). *Leptospira* serovars have also been detected among human and wild small mammals in Misenyi District, Kagera (Mgode et al. 2019). The presence of serovars among humans and animals in Rukwa, Katavi and Kagera regions provides a clue to *Leptospira* existence in north-western Tanzania, particularly in Kigoma and other unsurveyed districts of the Kagera Region. Despite *Leptospira*'s presence in neighbouring regions and the engagement of communities in risk occupations, there is lack of data on leptospirosis seroprevalence and its predominant serovars among humans and livestock in Ngara and Kibondo districts. Therefore, the present study aimed to assess the prevalence and seroepidemiology of *Leptospira* serovars existing among humans and livestock in Kibondo and Ngara districts in North Western Tanzania with the prediction that *Leptospira* serovars are circulating between interacting interfaces on livestock and human beings.

Materials and Methods

Study area and sites

This study was conducted in Ngara and Kibondo districts (Figure 1). Ngara District is located in the Kagera Region about 1,800 m above sea level at 30° 45'S and 30° 40'E (KRIG 2019). The estimated population size of the district is 320,056 people (NBS 2012). The primary economic activities in the Ngara District include agriculture (coffee and banana farming), large-scale livestock keeping and tourism. Like other districts in the Kagera Region, Ngara has a tropical climate characterized by bimodal rainfall between October to November and March to May, and its mean annual rainfall is about 900 mm (ranging between 600–1,200 mm). The mean annual temperature is 21 °C (ranging from 20 to 30 °C). In Ngara District, the study sites were Nyamiaga District Hospital, Mabawe village, Kabanga village and Murukukumbo village.

Kibondo District, on the other hand, is one of the six districts of the Kigoma Region. It is located in north-western Tanzania at 4° S and 31° E, 1254 m from the sea level. The estimated population size of the Kibondo District is 261,331 people (NBS 2012). The district receives an annual mean rainfall of about 900 mm (600–1,000 mm) and an average temperature of 24.6 °C (KRSEP 2016). The main economic activities in the Kibondo District include agriculture, livestock keeping and honey production. The study sites in Kibondo were Kibondo District hospital, Minyinya village, Kifura village, Kitahana village and Twabagondozi village.

Sampling design

Blood was drawn from humans and livestock (i.e. cattle and goats) residing in the same locality to ensure capturing of circulating strain. Within each village, humans who visited health facilities in their respective villages were included upon their consents after sensitization. Cattle and goats sampled for blood screening shared the same surroundings as screened humans, with the assumption that their interactions may influence the pathogens' transmission loop. Furthermore, the study used cattle and goats sharing the same grazing area as infections may originate from the source (i.e. grazing and water-drinking points). Before the blood drawing exercise, a local meeting was organised in each health facility to sensitize

participants and broaden their awareness on zoonoses, pathogen reservoirs, their associations with their socio-economic activities, and their impacts. After the sensitization, participants were asked for consent; they signed the consent forms per guidelines and rules on collecting samples from humans as stipulated on the permit issued by the National Institute of Medical Research (NIMR) No. NIMR/HQ/R.8a/Vol.IX/2951. Blood samples were also collected from humans who did not visit the hospitals, dispensaries or health centres but engaged in livestock keeping or slaughtering or frequently used animal products, including meat, milk and skins processing. Murukukumbo village has no health facility. Due to their high interactions with their livestock, livestock keepers requested to be included in the study, in which a special arrangement with the Authority of Kabanga Dispensary was made following protocols stipulated in sampling guidelines. Human blood samples were drawn by laboratory technicians assigned by the District Health Committee within their health facilities. A qualified veterinary officer collected blood samples from livestock at the district level with Ward Livestock Field Officers (LFOs).

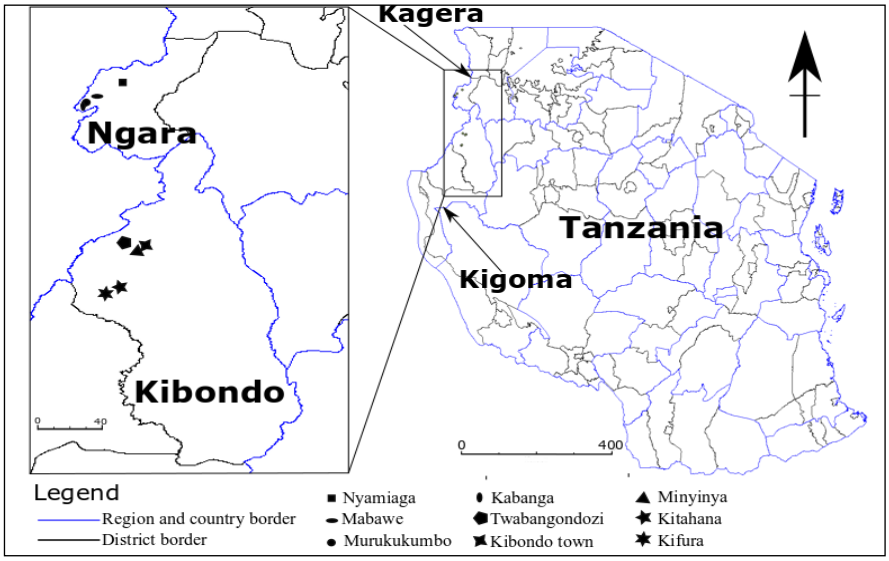

Figure 1: Map of Tanzania showing the study sites for investigating Leptospirosis in selected areas of Kigoma and Kagera regions.

Sample size

The standard equation determined the number of human individuals involved in the study for the unlimited population size (Kasiulevičius et al. 2006), $n = \frac{Z^2 \times P(1-P)}{d^2} + D$ in which:

n = estimated sample size; Z = standard normal variant for 95% Confidence Level (CL); P = population proportional; d = precision/margin of error; D = Power of study. For our study, Z = 1.96; P = 10% (0.1); d = 0.05; D = 15% of n. Based on the formula above, the estimated population size for this study was 159 human participants. The human samples were increased to 440 people to ensure more accuracy.

Livestock sample size: Estimated by using the following formula $n = \frac{Z^2 \times P(1-P)}{d^2} + D$ in which n = estimated sample size; Z = standard normal variant for 95% Confidence Level (CL); P = isolation success rate; d = precision/margin of error; D = Power of study. For our study, Z = 1.96; P = 8% (0.08); d = 0.04; D = 15% of n. Based on the formula above, the estimated population size for this study was 204 livestock (cattle and goats). The livestock samples were increased to 242 animals to increase accuracy.

Assessment of awareness and risk factors associated with the spread of leptospirosis

The awareness towards leptospirosis and risk factors associated with the disease were assessed using structured and unstructured questionnaires. The questions were based on demographic information, existing knowledge of leptospirosis and their reservoirs, known cases of leptospirosis infections, perception of leptospirosis infections, preventive and control measures, and effectiveness of the current control measures, public interventions, and accessibility to public health facilities. The risk factors associated with transmission of leptospirosis were assessed by associating the results of sera with the human activities of the participants. The major human activities considered included handling animals without protective gear, farming in contaminated soil, fishing in contaminated water, drinking contaminated water, feeding contaminated food, direct skin contact with the urine of the reservoir host, and consuming contaminated vegetables not washed thoroughly or half cooked. The selection of these parameters was based on the lifestyles of participants within the studied ecosystem (Figure 2).

Blood samples collection and handling

Blood samples were drawn from humans and livestock (i.e. cattle and goat) using vacutainers and labelled with specific codes. Blood samples were drawn from humans and livestock by laboratory technicians assigned by the District Health Committee and a qualified veterinary officer in conjunction with Ward Livestock Field Officers (LFOs), respectively (*sensu* Mgode et al. 2019). Human blood was collected from hospitalized and non-hospitalized patients who visited their villages' dispensaries, health centres and hospitals. The livestock (i.e. cattle and goats) were restrained before drawing blood from jugular veins. Blood (12 mL) was drawn from each volunteering human and animal using a vacutainer tube (i.e. anticoagulant-free) and left to settle for twelve hours, after which the supernatant (serum) was collected and kept in a well-labelled Eppendorf tube. The serum was then stored in dry ice before being transported to the Department of Zoology and Wildlife Conservation, University of Dar es Salaam and frozen at -80 °C before analysis.

Figure 2: mpressions of interactions that may cause transmission of leptospires between humans and livestock in Ngara and Kibondo Districts. Images **A** and **B** show the possibility of contamination of soils and vegetables in the grazing areas. Images **C** and **D** show improper livestock handling that exposes the possibility of acquiring spillovers of waste that can be sources of transmissions of pathogens. Images **A** and **D** were taken in Twabagondozi village (Kibondo), while Plates **B** and **C** taken in Murukukumbo village (Ngara), respectively. (Photo credit: J.V. Katandukila & Y.J. Chuhila, December 2019).

Microscopic agglutination test for detection of *Leptospira* antibodies

The presence of *Leptospira* antibodies was screened by Microscopic Agglutination Test (MAT) using a panel of live reference and local isolate serovars belonging to seven serogroups commonly found in Tanzania. These included Sokoine (*Leptospira kirschneri)*, Kenya (*Leptospira borgpetersenii*), Sejroe (*Leptospira interrogans*), Grippotyphosa (*Leptospira kirschneri*), Bataviae (*Leptospira interrogans*), Lora (*Leptospira interrogans*) and Pomona (*Leptospira interrogans*). Live bacteria of the 7 serovars above were cultured in Ellinghausen and McCullough–Johnson and Harris, (EMJH) culture medium at 30 °C for seven days. The antigen cultures were checked for adequate density (3 x 10^8 cells/ml) and absence of contamination by using a dark field microscope. Briefly, MAT was performed by diluting 10 µL of serum in a microtitre plate containing 90 µL of PBS in the wells of the first row (1:10) dilution. Then, a serial dilution was done in the columns of the remained wells that were previously filled with 50 µL of PBS. After serial dilution, 50 µL of the live *Leptospira* antigen was added to each microtitre plate well. This doubled the dilution consecutively from the first row at 1:20 to the last row. Then, the plates were shacked by hand for one minute and incubated at 30 °C for 2 hours. The serum–antigen mixture was examined under a dark field microscope to observe the agglutination by taking a drop of mixture from the wells of the microtitre plate to a microscopic slide and examined under the dark field microscope using X20 objective. A positive sample was noted by detecting 50% *Leptospira* agglutination and living 50% of cells free. This was compared with a negative control suspension of leptospires diluted 1:20 in PBS.

Data analysis

The information from questionnaires and interviews were subjected to Statistical Package for Social Sciences (SPSS) version 21 (IBM Corporation 2013). The selected demographic information and extent of awareness of leptospirosis were presented as a percentage. The logistic regression model evaluated the strengths of risk factors for associated socio-economic activities on leptospirosis infections, assuming that socio-economic activities may expose humans to infections or no condition.

Quantitative data were subjected to R algorithm version 3.4.2 (R Core Team 2017). The Shapiro Wilk test evaluated the data's normality ($W = 0.728$, $p = 0.307$). The seropositivity of *Leptospira* serovars and seroprevalence of leptospirosis presented as a percentage. Chi-square statistical tests determined comparisons of variables. All statistical tests were significant at $\alpha = 0.05$.

Ethical approval

The study was approved by the University of Dar es Salaam (Certificate No. CoNAS-ZLW18058), the National Institute of Medical Research (NIMR: No. NIMR/HQ/R.8a/Vol.IX/2951) and the Commission for Science and Technology (No. 2019-84-NA-2018-387). The study was also approved by the management of Ngara and Kibondo districts (HW/A/E.10/151/1 and HW/A/E.50/4/07, respectively).

Results

Awareness of participants on leptospirosis

Ninety percent (90%) of respondents (n = 600) understood zoonotic transmitted diseases, of which females were 59.3% and males presented 30.7%. Zoonotic diseases of great awareness in the ecosystem were sarcoptic mange (53.8%), lyme (23.1%), rabies (11%), plague (7.2%), brucellosis (3.5%) and leptospirosis (1.4 %). Among the avenues for awareness of zoonotic diseases media contributed the highest percentage (67.72%) followed by sensitization of veterinary officers (22.03%) and school education by 5%. The awareness of the preventive measures on zoonoses, including leptospirosis, showed 4%, while unawareness showed 96%.

Assessment of risk factors associated with transmission of leptospirosis

Among the selected human activities, drinking contaminated water and consuming contaminated food showed the highest odds ratio of 7.40; 95% CL = 1.3046–50.4761 and 6.10; 95% CL = 1.9034–13.2482, respectively (Table 1). Eating contaminated vegetables that were not washed thoroughly and half-cooked showed the lowest odds ratio values of 1.44; 95% CL = 0.9016–2.0620. Comparison of odds ratio of human activities related to the transmissions of leptospirosis between districts varied insignificantly ($\chi^2_{(1)}$ = 1.094, p = 0. 2956) while the variation of odds ratios between risk activities was significant ($\chi^2_{(6)}$ = 17.769, p = 0.00685).

Table 1: Odds ratio and critical values of selected human activities in association with infections of leptospirosis in Ngara and Kibondo Districts. CL = confidence interval; Data collected in the years 2018–2019).

Human Activity	Odds ratio	P-value	95% CL
Direct skin contact with the urine of the reservoir host	3.19	0.0010	1.7582–5.6116
Consuming contaminated vegetables not washed thoroughly and half cooked	1.44	0.2124	0.9016–2.0620
Drinking contaminated water	7.40	<0.0001	1.3046–50.4761
Eating/Feeding contaminated food	6.10	0.0014	1.9034–13.2482
Farming in contaminated soil	5.14	<0.0001	2.3953–11.0305
Handling animals without protective gears	1.96	0.0101	0.4475–8.5708
Fishing in contaminated water	1.80	0.1003	0.6536–5.3788

Seropositivity of sera against *Leptospira* serovars in north-western Tanzania

Of all the tested sera (n = 682), 163 sera (23.9%) were positive for *Leptospira* serovars at titre dilution of ≥ 1:20 in which Ngara District had seropositivity of 12.8% (87 sera) and Kibondo District had 12.6% (86 sera). Sera agglutinated at ≥ 1:160 accounted for 59 positive sera (13.4%) in which Ngara District had 33 positive sera (7.5%) and Kibondo District showed 26 positive sera (5.9%). The titre dilution of 1:320 was positive for seven sera (1.6%) in which Ngara District had two positive sera (0.6%), and Kibondo District showed five positive sera (1.1%). The dilution titre of 1:640 showed four positive sera (0.9%), in which Ngara District had three and Kibondo District had one positive serum, respectively.

Among the positive sera (163 sera), the seropositivity of 33.7% was recorded against Sokoine *Leptospira* serovar in which Ngara District had 17.2% (28 sera), and Kibondo District had 16.6% (27 sera). The Sejroe and Grippotyhposa *Leptospira* serovars showed seropositivity of 20.9% (34 sera) and 20.2% (33 sera), respectively (Ngara District, Sejroe = 10.4%; Grippotyphosa = 9.2%: Kibondo District, Sejroe = 10.4%; Grippotyphosa = 11.0%). *Leptospira* serovar Kenya showed seropositivity of 9.2% (15 sera), in which Ngara District showed 5.5%, while Kibondo District had 3.7%. *Leptospira* serovars Pomona and Lora had seropositivity of 6.1% each (Ngara District, Lora = 4.9%; Pomona = 4.3%: Kibondo District, Lora = 1.2%; Pomona = 1.8%) with Batavia *Leptospira* serovar showed 3.7% (6 sera) whereas Ngara District showed 1.8% and Kibondo District had 1.8%. The variation of seropositivity between tested *Leptospira* serovars was significant ($\chi^2_{(6)}$ = 18.274, p = 0.0056) in which Sokoine *Leptospira* serovars had the highest seropositivity followed by Sejroe and Grippotyphosa. Seropositivity of *Leptospira* serovars was insignificant between districts ($\chi^2_{(1)}$ = 3.241, p = 0.0718). In Ngara District, two sera were multi-positive for both Grippotyphosa and Lora *Leptospira* serovars, and one serum was positive for *Leptospira* serovars Lora, Pomona and Kenya. In the Kibondo District, multi-seropositivity of Grippotyphosa and Kenya *Leptospira* serovars was recorded in four sera, with one

serum being positive for both Sejroe and Bataviae *Leptospira* serovars.

Seroprevalence of leptospira serovars in human

Seroprevalence of 11.1% (n = 682) detected in human sera whereas Ngara District showed seroprevalence of 6.0% and Kibondo District had a seroprevalence of 5.1%. Among the human sera (n = 440), Ngara District (n = 220; 109 females and 111 males) had a seroprevalence of 18.6%, while Kibondo District (n = 220; 105 females and 115 males) had a seroprevalence of 15.9%. In Ngara District, the seroprevalence of 6.8% and 5.5% were recorded in human sera from Mabawe and Kabanga health facilities, while in Kibondo District seroprevalence of 4.1% and 3.6% were recorded in human sera from Minyinya and Twabagondozi health facilities (Table 2). Comparison of seroprevalence between districts ($\chi^2_{(1)}$ = 2.546, p = 0.1106) and health facilities within districts varied insignificantly (Ngara District: $\chi^2_{(3)}$ = 7.486 , p = 0.0579; Kibondo District: $\chi^2_{(4)}$ = 6.006, p = 0.1987). Females had seroprevalence of 10% with males had seroprevalence of 7.3%, whereas in Ngara District, female had 11.4% and male had 7.3% with Kibondo District seroprevalence was 8.6% and 7.3% for females and males, respectively. The variation of seroprevalence between sexes was significant ($\chi^2_{(1)}$ = 4.010, p = 0.0452) with females' sera showed higher seroprevalence.

Table 2: Seroprevalence of *Leptospira* in patients and non-patients in Ngara District (n = 220) and Kibondo District (n = 220); n = screened sera; N = positive sera

District	Health facility	Seroprevalence (%)			
		Pooled sexes (within districts)	Within health facility	Females	Males
Kibondo	Minyinya dispensary (n = 44)	3.6	18.2 (N = 8)	6.8	11.4
	Kifura health center (n = 44)	2.3	11.4 (N = 5)	6.8	4.5
	Kitahana dispensary (n = 44)	3.2	15.9 (N = 7)	11.4	4.5
	Twabagondozi dispensary (n = 44)	4.1	20.5 (N = 9)	11.4	9.1
	Kibondo hospital (n = 44)	2.7	13.6 (N = 6)	9.1	4.5
Ngara	Mabawe dispensary (n = 55)	6.8	27.3 (N = 15)	16.4	10.9
	Kabanga dispensary (n = 55)	5.5	21.8 (N = 12)	14.5	7.3
	Nyamiaga hospital (n = 55)	2.3	9.1 (N = 5)	5.5	3.6
	Murukukumbo village* (n = 55)	4.1	16.4 (N = 9)	9.1	7.3

* = It is not a health facility but blood collected following stipulated protocols in the permit no. NIMR/HQ/R.8a/Vol.IX/2951. (Data collected in year 2018–2019).

***Leptospira* seroprevalence among livestock**

Livestock sera had a seroprevalence of 12.8% (n = 682) in which seroprevalence of 6.2% and 6.6% was recorded in goats and cattle, respectively. Among livestock (n = 242), goats had a seroprevalence of 17.4%, Ngara District 9.1%, and Kibondo District 8.3%. Goats sera were positive for five *Leptospira* serovars in both districts: Sokoine, Sejroe, Grippotyphosa, Pomona and Bataviae. Among the positive sera of goats (42 sera), *Leptospira* serovars Sokoine and Sejroe showed a seroprevalences of 45.2% and 21.4%, in which Ngara District had seroprevalences of 50% and 22.7% while Kibondo District had seroprevalences of 40% and 20%, respectively (Table 3). The *Leptospira* serovar Bataviae showed lowest seroprevalence of < 10% in both districts.

Cattle had a seroprevalence of 18.6%, Ngara district 9.9%, and Kibondo 8.7%. Cattle sera were positive for four *Leptospira*

serovars in both districts: Sejroe, Grippotyphosa, Pomona and Bataviae. Among the positive sera of cattle (n = 45 sera), *Leptospira* serovars Sejroe showed a seroprevalence of 44.4% with *Leptospira* serovars Kenya and Grippotyphosa had seroprevalence of 22.2% (Table 3). The *Leptospira* serovar Lora showed seroprevalence of 11.1%. One serum of cattle co-agglutinated with Kenya and Grippotyphosa *Leptospira* serovars. Seroprevalence of *Leptospira* serovars varied insignificantly between districts ($\chi^2_{(1)}$ = 3.812, p = 0.0509) as well as between goats and cattle ($\chi^2_{(1)}$ = 2.942, p = 0.0863); however, Ngara District had more *Leptospira* serovars than Kibondo District and goats had more *Leptospira* serovars than cattle despite high seroprevalence in cattle.

Table 3: Seroprevalence of *Leptospira* serovars in livestock (i.e. goats and cattle) in Ngara and Kibondo districts, north-western Tanzania. Numbers in parentheses = sample size. (Data collected in the year 2018–2019).

Leptospira serovars	Seroprevalence (%)					
	Ngara District			Kibondo District		
	Livestock pooled (121)	Goats (61)	Cattle (60)	Livestock pooled (121)	Goats (60)	Cattle (61)
Sokoine	23.9	50.0	0	19.5	40.0	0
Grippotyphosa	15.2	9.1	20.8	22.0	20.0	23.8
Lora	6.5	0	12.5	4.9	0	9.5
Kenya	13.0	0	25.0	9.8	0	19.02
Pomona	4.3	9.1	0	7.3	15.0	0
Sejroe	32.6	22.7	41.7	34.1	20.0	47.6
Bataviae	4.3	9.1	0	2.4	5.0	0

Cyclic serovars among humans, goats and cattle in north-western Tanzania

The seropositivity of *Leptospira* serovars Sejroe and Grippotyphosa was recorded in humans, goats and cattle sera (Figure 3). The humans and goats had seropositivity with *Leptospira* serovars Sokoine, Pomona and Bataviae, while Kenya and Lora serovars were positive in the sera of cattle and humans. Humans and goats shared 5 *Leptospira* serovars, while humans and cattle shared 4 *Leptospira* serovars. Goats and cattle shared 4 *Leptospira* serovars (Figure 3). The variations of seroprevalance of *Leptospira* serovars between humans, cattle and goats were significant ($\chi^2_{(2)}$ = 7.9991, p = 0.0183), but insignificant between goats and cattle ($\chi^2_{(1)}$ = 1.942, p = 0.1635).

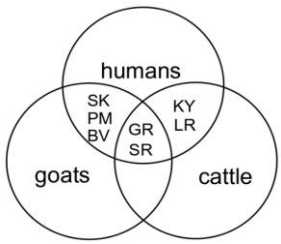

Figure 3: The relationships of *Leptospira* serovars (SK = Sokoine; PM = Pomona; BV = Bataviae; GR = Grippotyphosa; SR = Sejroe; KY = Kenya; LR = Lora) among cattle, goats and humans in Ngara and Kibondo districts, north-western Tanzania. (Data collected in the year 2018–2019).

Discussion

The present study showed the prevalence and circulation of *Leptospira* pathogens among humans and livestock in the Ngara and Kibondo districts. The high prevalence of leptospirosis in the study area might be attributed to the high interactions of human

activities that enhance contacts with potential leptospirosis reservoir hosts. Previous studies suggested that human activities such as livestock keeping, farming in wetland areas, fishing or working in areas inhabited by rodents play significant roles in transmitting pathogens to humans (Benacer et al. 2016, Mgode et al. 2019, 2021). As reported in similar studies, livestock such as goats and cattle might be leptospirosis reservoir hosts or acquired *Leptospira* serovars from other hosts, including rodents (Assenga et al. 2015, Mgode et al. 2021, Msemwa et al. 2021).

Drinking contaminated water and consuming contaminated food are risk factors with the highest chances of ingesting *Leptospira* pathogens. In rural areas, the treated water supply is limited; thus, local communities use untreated water from ponds, dams and wells filled with rain run-off contaminated by humans and non-human animals. Farming activities also scored high odds ratio because various non-human animals use farming pads as feeding sites in which their excreta may be sources of leptospirosis as reported that soils is a potential source of leptospirosis transmission (Bierque et al. 2020). Feeding contaminated vegetables that are not washed through and half cooked is also a risk factor, especially when making a salad, and those who prefer half-cooked vegetables without considering the contact interface between gardens, storage facilities and preparatory hygiene. Contrary to Mgode et al. (2019) who reported fishing as the highest risk factor for transmission of leptospirosis in a similar study area, the present study reports the lowest risk of fishing activities in the Ngara District, Kagera Region. The variation is attributed to less priority on fishing as a socio-economic activity due to few water bodies; in turn, farming and domestication of animals are major socio-economic activities. Despite water bodies in the Kibondo District (KRSEP 2016), people engaged in farming and livestock keeping; thus, fishing has less risks of transmitting *Leptospira* pathogens. The susceptibility of human activities as risk factors for the transmissions of leptospirosis corroborates with other studies (Allwood et al. 2014, Assenga et al. 2015, Mgode et al. 2019, 2021, Bierque et al. 2020, Msemwa et al. 2021).

Little awareness of leptospirosis among the residents in the Kibondo and Ngara districts may have resulted from less publicity and the lack of specific symptoms. Most health agendas entail strategic epidemic diseases with little attention on leptospirosis, which signifies that it is a disease of little attention. Interestingly, little understanding of leptospirosis was even among healthcare professionals. Leptospirosis has been reported to manifest symptoms of highly publicized diseases, including malaria, typhoid, hepatitis, Lassa fever, dengue, yellow fever, tuberculosis, Urinary Tract Infection (UTI) and brucellosis (Biggs et al. 2011). The co-symptomatic leptospirosis with common diseases may cause fewer interventions of *Leptospira* diagnostic tools in health facilities due to irrational information on its impacts on the ecosystems. The little understanding of leptospirosis may increase the outbreak's impacts due to limited knowledge of transmission modes and preventive measures. The effects of less understanding of neglected diseases, including leptospirosis, have been reported to cause massive deaths when outbreaks occur in various places (Wiwanitkit 2006, Allwood et al. 2014, Ricardo et al. 2018).

The present study revealed the presence of seven *Leptospira* serovars Sokoine, Kenya, Sejroe, Grippotyphosa, Bataviae, Pomona and Lora. The titre dilutions of reactivity of these *Leptospira* serovars indicate that leptospirosis is prevalent within the study area. The multi-positive of antibodies against *Leptospira* serovars intimate that more than one strain of *Leptospira* serovars may infect a single host. The reactivity of *Leptospira* serovars Sokoine, Sejroe and Grippotyphosa signify their commonness and thus can be acquired. The trend of prevalence of these *Leptospira* serovars concurs with reports of other studies within north-western Tanzania (Biggs et al. 2011, Assenga et al. 2015, Mgode et al. 2019). The predominance of *Leptospira* serovars Sejroe and Grippotyphosa was reflected by its high

seroprevalence in humans, goats and cattle. The *Leptospira* serovar Sokoine is highly prevalent in north-western Tanzania, but uninfected cattle although co-infected humans and goats as reported elsewhere by Gregory et al. (2018). Pomona, Bataviae, Lora and Kenya, were prevalent in humans. However, goats lack the latter two serovars, and cattle lack the first two serovars indicating the possibility of serovars specificity in livestock despite sharing foraging areas. Humans seem to acquire all *Leptospira* serovars as they interact with all susceptible interfaces, including contaminated soils, water, food, contacting animal manure, use of products of affected livestock and other hosts, including rodents. The sharing of *Leptospira* serovars between human, goat and cattle signify the impacts of human-livestock interactions, which poses pathogens spread between interacting interfaces. The cycling of *Leptospira* serovars between human and non-human animals may be prompted by sharing drinking sites, touching spillovers of host excreta such as urine, saliva and faecal of affected animals and interactions in gardens and farming pads. Similar results on the cycling of *Leptospira* between humans and livestock have also been reported in other studies (Assenga et al. 2015, Mgode et al. 2021, Msemwa et al. 2021).

Conclusion and Reccommenations

Kibondo and Ngara districts in north-western Tanzania have prevalence of *Leptospira* serovars Sokoine, Kenya, Sejroe, Grippotyphosa, Bataviae, Pomona and Lora. The co-infections of *Leptospira* serovars were recorded in which hosts (human, goat and cattle) were infected by more than one serovar. Sharing of similar *Leptospira* serovars was recorded in humans, goats and cattle, intimating cyclic serovars between interfaces as a consequence of their interactions. Risk factors associated with the transmissions of leptospirosis include contacting a contaminated environment and touching of excreta of affected animals. Given the observed prevalence from this study, a call for health interventions, educational programmes on the modes of transmissions and avoidance of leptospirosis is highly recommended. The extensive research may include a larger sample size of both interfaces as well as the inclusion of wild animals. Multidisciplinary analysis, including molecular analysis, is suggested to unveil the phylogenetic relationships of serovars between hosts because MAT cannot reveal the causes of overlaps and divergence immune relationships among livestock as they share few *Leptospira* serovars although they share foraging and dens.

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