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The Prevalence of *Leptospira* Serovars in African Giant Pouched Rats (*Cricetomys* spp.) from the Ngorongoro Conservation Area, Tanzania

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Simple Summary: This study has investigated the presence of *Leptospira* bacteria in urine, kidney, and blood samples from African giant pouched rats (*Cricetomys* spp.) in the Ngorongoro Conservation Area (NCA), Tanzania. The giant rat is an important maintenance host of leptospires; understanding their abundance and distribution in this environment is important to help prevent the transmission of the bacteria from rodents to other animals and humans co-existing in the area. This study demonstrates that *Cricetomys* spp. in the NCA are infected with the spirochete, and are hence a potential threat to public health. This study calls for an enhanced awareness of the presence of *Leptospira* spp. in *Cricetomys* spp. and calls for a one-health approach to protect both humans and animals from the pathogens.

Abstract: Leptospirosis, also known as Weil's disease, is a febrile tropical disease of humans and diverse animals. The maintenance hosts of the infectious pathogen, *Leptospira* spp., are primarily rodents, while other warm-blooded animals and some reptiles are secondary or transient hosts of this pathogen. African giant pouched rats (*Cricetomys* spp.) have been identified to be important maintenance hosts of pathogenic leptospires in the tropical and subtropical regions of the world. This study assessed the seroprevalence of *Leptospira* spp. in the African giant pouched rats of the Ngorongoro Conservation Area (NCA), Tanzania, where there is close human, domestic animal, and wildlife interaction. A total of 50 African giant pouched rats were sampled between July 2020 and December 2021. Blood sera were screened for specific leptospiral antibodies using a microscopic agglutination test (MAT), while urine and kidney tissues were examined for the pathogen and pathogen-specific genes using cultures and polymerase chain reactions (PCR), respectively. The pathogen detection varied from 0% in cultures to 6% via the MAT and 20% via PCR. The Fisher exact test was applied to compare positive cases detected through the diagnostic tests, and showed a significant difference in the indirect and direct detection of *Leptospira* serovars via the MAT and PCR. We conclude that pathogenic *Leptospira* serovar are found in the NCA and recommend that the NCA authority raises awareness of the existence of the *Leptospira* serovar in giant African pouched rats, and possibly other rodents. The NCA should initiate appropriate management strategies, including the guided disposal of household garbage, which is the major attractant of rodents to residential areas. Where necessary, the NCA should carry out limited rodent control and periodic monitoring of the pathogen carrier (rodent) populations.

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1. Introduction

Leptospirosis, also known as Weil's disease, is a zoonotic disease with significant morbidity and high mortality rates whenever outbreaks occur. The average annual morbidity and mortality rates worldwide are estimated to be 14.77 and 0.84 cases per 100,000, respectively [1]. Leptospirosis is a febrile neglected tropical zoonotic disease with worldwide distribution in diverse mammalian hosts, including rodents, shrews, cattle, and wildlife [2–4]. Maintenance hosts are typically asymptomatic; however, accidental hosts, such as humans, may experience a variety of clinical manifestations, including serious pulmonary hemorrhage, hepatic and renal failure, and even death [5]. Rodents are the primary reservoir hosts for various *Leptospira* serovars, including pathogenic strains. They maintain and spread leptospires in the environment, which can lead to human and other animal infections through contact with contaminated soil, water, and food sources. Several rodent species, such as *Rattus* spp., *Mus musculus*, *Bandicota bengalensis*, *Bandicota indica*, and *Cricetomys* spp., are known to carry different pathogenic leptospiral serovars and exhibit host specificity for certain serovars, such as Icterohaemorrhagiae, Copenhageni, Ballum, Lora, Qunjian, and Australis, among others. *Mus musculus*, *Rattus* spp., and *Bandicota* spp. have been identified as hosts to several *Leptospira* spp. strains, including *L. borgpetersenii*, *L. kirschneri*, and *L. interrogans*, in research conducted in many parts of the world, like the US, Malaysia, Bangladesh, and Australia [6–9]. *L. borgpetersenii* has been detected in *Mus musculus* and *L. interrogans* in *Rattus* spp. in Angola [10]. *L. interrogans* and *L. kirschneri* are carried by *Mus* populations in Kenya [11]. *Cricetomys* spp. carry different *Leptospira* spp. strains such as *L. interrogans* serogroup Ballum and serogroup Australis in Tanzania [12–14]. *Leptospira* serovar colonize the renal tubules and urogenital tracts of the host; from there, they are excreted with urine into the environment [15]. *Leptospira* can stay for several months in wet conditions [16]. Contaminated water bodies and soils are, therefore, major sources of *Leptospira* bacteria infecting humans and susceptible animals [17]. The disease manifests asymptomatically in maintenance hosts; however, accidental hosts, such as humans, may experience a variety of clinical manifestations, including serious pulmonary hemorrhage, hepatic and renal failure, and even death [18]. Diagnostic tools that are commonly employed for leptospirosis include the microscopic agglutination test (MAT), considered the reference serological standard assay, and the enzyme-linked immunosorbent assay (ELISA) [19]. The MAT is the reference method for serological diagnosis, and is primarily used in epidemiological studies, as it can identify the most prevalent leptospiral serovars or serogroups [20]. While cross-reactivity can occur, the MAT provides valuable information on the epidemiology of leptospires. The test detects both IgM and IgG antibodies, making it useful for epidemiological studies [21].

Polymerase chain reactions (PCRs) are the standard molecular diagnostic tests for leptospirosis. They are favored for their speed, reliability, and ability to detect early infections and distinguish between pathogenic and saprophytic leptospiral serovars [22].

Cricetomys spp., commonly known as giant rats, are acknowledged as essential maintenance and reservoir hosts of pathogenic leptospires due to their burrowing behavior and preference for domestic garbage dump sites. They may carry and shed various leptospiral serovars, contributing to the environmental contamination of habitats shared with other mammals, including humans, domestic animals, and wildlife [2,23,24]. According to recent studies [25,26], most species of the genus *Leptospira*—family *Leptospiraceae*—are saprophytic. Habitats that humans and domestic animals share with rodents for food and shelter, particularly those that are close to human habitation, improper waste management sites, and wet environments, have been identified as significant sources of *Leptospira* infection for humans and domestic animals [27]. Several studies on *Leptospira* seropositivity in rodents have been carried out in many districts in Tanzania, including Katavi, Morogoro, Kilimanjaro,

and Tanga [13,14,28,29]. A study in central Tanzania recommended the investigation of the effect of climatic change on rodent populations known to be *Leptospira* carriers [17]. The prevalence of *Leptospira* in important maintenance hosts has, however, been under-studied in areas such as the NCA, which is one of the world's most iconic natural conservations with a unique, unrestricted interaction between humans, livestock and wildlife. Habitats that humans and domestic animals share with rodents for food and shelter, particularly close to human habitation, improper waste management sites, and wet environments, have been identified as significant sources of *Leptospira* infection for humans and domestic animals [30].

Therefore, understanding the presence of the *Leptospira* serovars circulating in their natural reservoir hosts in the NCA is important for creating awareness of the leptospires pathogen that will help to minimize the risks of transmission among the animals and humans living in the area. Furthermore, investigating leptospira serovars in *Cricetomys* spp. is crucial for understanding the prevalence of leptospira serovars among the natural and incidental reservoirs.

In this study, we report the presence and distribution of the African giant pouched rat in the NCA, and the prevalent pathogenic leptospiral serovars in these rats. The study hypothesizes that leptospiral serovars are prevalent in *Cricetomys* spp. in the NCA forests and around human settlements, including tourist hotels. To support this hypothesis, researchers collected blood, urine, and kidney tissue samples from *Cricetomys* spp. for leptospiral isolation, using culture and serology by microscopic agglutination test (MAT). In addition, DNA from the kidney tissues was examined for leptospira-specific genes using polymerase chain reaction (PCR). Understanding the presence of *Leptospira* in these rodents is not only essential for their conservation but also for understanding the risk of disease transmission from rodents to other animals that coexist within the NCA, as it has been known that this area is shared between wildlife, livestock, and humans.

2. Materials and Methods

2.1. Study Sites

This study was carried out in Ngorongoro Conservation Area (NCA), located in the Arusha region, northern Tanzania, situated between longitude 34°52′ and 35°58′ E and latitude 2°30′ and 3°38′ S. The NCA is a unique environment with a multiple land-use system that allows wildlife conservation, community farming, the grazing of livestock by pastoralists and tourism [31,32]. The conservation area has moist and misty conditions, with annual temperatures ranging from 35 °C in February to 2 °C in June/July [33]. Rainfall is seasonal and variable, ranging from 400 to 600 mm/year in the arid lowland plains in the west, while in the Eastern Highland forested areas, rainfall varies from 1000 to 1200 mm/year [34]. The NCA has a bimodal seasonal variability with two wet and two dry seasons. The wet periods are from October to December and March to May. A short dry season extends from January to February while a long dry period is between June and September [35].

2.2. Rodent Trapping and Sampling

Purposive convenient sampling was carried out in which rodents were sampled at the time of capture [36]. Traps were set around the human settlement and in four selected vegetated areas (forest, woodland, bushland, and grassland). These sites were selected as they were suitable for rodent harborage. Five line transects of 100 m each were created. For each transect 10 traps (60 × 15 × 170 cm) (Havahart® collapsible cage trap, Easy Technology Solutions Limited, Auckland, New Zealand) were set 10 m apart, making a total of 50 traps for each of the four types of vegetation [37]. The transects were set parallel to each other and the distance from one transect to another was 25 m [38]. A total of 50 traps were randomly set around human settlements (staff homes and tourist lodges) [39]. Each trap was baited with a mixture of avocado, ripe bananas, and green maize, left overnight, and inspected once daily every following morning before 08:00 h. The trapping was carried

out between July 2020 and December 2021 for six sessions at each site, each session lasting 10 consecutive nights.

The captured rodents were humanely killed by excess inhalation of halothane-soaked cotton wool. Immediately after death, cardiac puncture was performed after treating the ventral part of the animals with 70% ethanol. Blood was placed in 2.5 mL microcentrifuge tubes for serum preparation. The collected blood was allowed to clot, and thereafter centrifuged at 4000 rpm for 10 min. The obtained serum was stored at −20 °C before the MAT test. After blood collection, each rodent was aseptically dissected using sterilized scissors and forceps then urine from the urinary bladder was collected using a sterile syringe and needle. Thereafter, the kidney was aseptically sectioned and a piece was placed into a sterile 5 mL glass tube containing 200 µL of phosphate-buffered saline for culture. A duplicate piece was placed into a microtube of 2.5 mL containing absolute ethanol for molecular analysis.

2.3. Detection of Leptospiral Antibodies by Microscopic Agglutination Test

The MAT, which is the serological gold standard assay in leptospirosis diagnosis, was used to detect antibodies against *Leptospira* serovar in the *Cricetomys* spp. serum [40]. Briefly, live leptospiral antigens of serovars Sokoine, Hebdomadis, Grippotyphosa, Lora, and Pomona were used. These serovars were selected based on previous studies performed to identify the circulating serovars in the northern part of Tanzania [28,41]. Sokoine has been isolated from cattle and rodents, Hebdomanis has been found in cattle, Grippotyphosa has been isolated from cattle, Lora has been isolated from rodents and Pomona has been isolated in cattle and buffaloes [14,17,42]. Hebdomanis, Grippotyphosa, and Pomona are found in cattle, and they have been included to check their availability in *Cricetomys* spp. Since in NCA, livestock, wildlife and humans interact closely, there is a possibility of transmission of *Leptospira* serovars.

The antigens were prepared in Ellinghausen and McCullough–Johnson and Harris (EMJH) [2] culture medium and checked for purity under a dark field microscope. Antigens with a density of 300×10^8 leptospires/mL and aged no more than 10 days were used in the test. The test was conducted by initially diluting 10 µL of serum with 90 µL of phosphate-buffered saline (PBS), pH 7.0, in a microtiter plate, followed by a serial doubling dilution into dilutions of 1:10, 1:20 1:40, and 1:80. Then, 50 µL of each antigen was added to the wells containing the diluted serum samples to achieve final dilutions of 1:20, 1:40, 1:80, and 1:160. For each serovar, a positive and negative control was set. The contents of the plates were homogenized briefly by manual shaking and then incubated at 30 °C for 2 h. The observation was performed under a dark field microscope (ZEISS, Cambridge, UK) at ×20 magnification. The agglutination titers were then evaluated and the cut-off titers were established visually as the point (titer) at which at least 50% of the leptospires exhibited agglutination compared to the negative control.

2.4. Molecular Characterization of Leptospira Serovars

2.4.1. DNA Extraction

DNA extraction of 50 kidney tissue samples was undertaken following the manufacturer's instructions (Zymo research kit—Quick-DNA Miniprep Plus Kit, Zymo Research Corporation, Irvine, CA, USA). Samples were prepared prior to extraction by exposing them to air (while still in cryovials to prevent contamination) to thaw. This was followed by rinsing with double-distilled water to ensure that no preservatives remained that could inhibit the action of reagents during extraction. After extraction, the DNA was introduced to a nanodrop spectrophotometer for quantification and then stored at −20 °C [3].

2.4.2. PCR Amplification, Gel Electrophoresis and Visualization

A 25 ul reaction mixture was prepared, consisting of 12.5 µL of Green Master mix (New England Biolabs, Ipswich, MA, USA), 0.5 µL (10 µM) each of Lepat 1 (5′-GAG-TCT-GGG-ATA-ACT-TT-3′) and Lepat 2 (5′-TCA-CAT-CG(YT)-GCT-TAT-TTT-3′); 8.5 µL of

nuclease-free water and 3 µL of DNA template [43]. PCR amplification involved specific primers targeting a 330 bp region of the 16 S rRNA gene (rrs) that was subjected to initial denaturation of the DNA at 93 °C for 3 mn, followed by 35 PCR cycles; final denaturation at 93 °C for 1 mn and primer annealing at 48 °C for 1 mn, followed by initial elongation at 72 °C for 1 mn and final elongation at 72 °C for 10 mn [2]. The PCR products were then loaded on 1.5% agarose gel, stained with GRGreen Nucleic Acid Stain (Excellgen, Inc., Rockville, MA, USA), and visualized on a Gel DocTM (Bio-Rad, Hercules, CA, USA) [44].

2.5. Culture

Urine and kidney homogenates were used for culturing, in which two drops of urine were placed in a 10 mL tube containing a selective growth inhibitor and 5 mL Fletcher medium (200 µg/mL) of 5-Fluorouracil, and then incubated at 30 °C. A piece of kidney tissue, previously placed into tubes containing 1 mL of PBS, was then immediately aseptically crushed using a sterile glass Pasteur pipette to obtain a homogenate. Then, after this, two drops of the homogenates were inoculated into a 10 mL tube containing Fletcher's medium as was undertaken for the urine (above) within 10 min, then they were incubated together at 30 °C for a minimum of 8 weeks while being examined weekly for leptospires growth by dark field microscopy [45,46].

2.6. Data Analysis

Microsoft Office Excel® 2016 was used for storing data and IBM SPSS version 26 (2019) was used for descriptive and inferential analyses [47]. The Fisher exact test was used to associate seroprevalence with trapping site and season. The confidence interval was set at a 95% limit and a *p*-value less than 0.05 was considered statistically significant [48].

3. Results

3.1. Abundance of Cricetomys spp. in Selected Sites Ngorongoro Conservation Area

A total of 50 *Cricetomys* spp. was captured, of which 49 were from near human settlements and one was found in the densely forested area TrS4 (Table 1, Figure 1). No captures were made in the woodland (TrS 2), bushland (TrS 3), or grassland (TrS 1). There was a significant difference in the numbers of captures between male and female *Cricetomys* spp., with male abundance (M = 4.71, S.D = 0.89) being greater than female (M = 2.43, S.D = 0.81) ($t(6) = 3.77$, $p = 0.009$) (Table 1).

Table 1. Distribution of *Cricetomys* spp. in NCA trap sites.

Site	Abundance	Male	Female
Kongoni	14	8	6
Ngorongoro Wildlife lodge	6	3	3
Pongo	11	7	4
Viewpoint	5	0	5
Lerai	7	5	2
Lemala	6	4	2
Forest	1	1	0
TOTAL	50	28	22

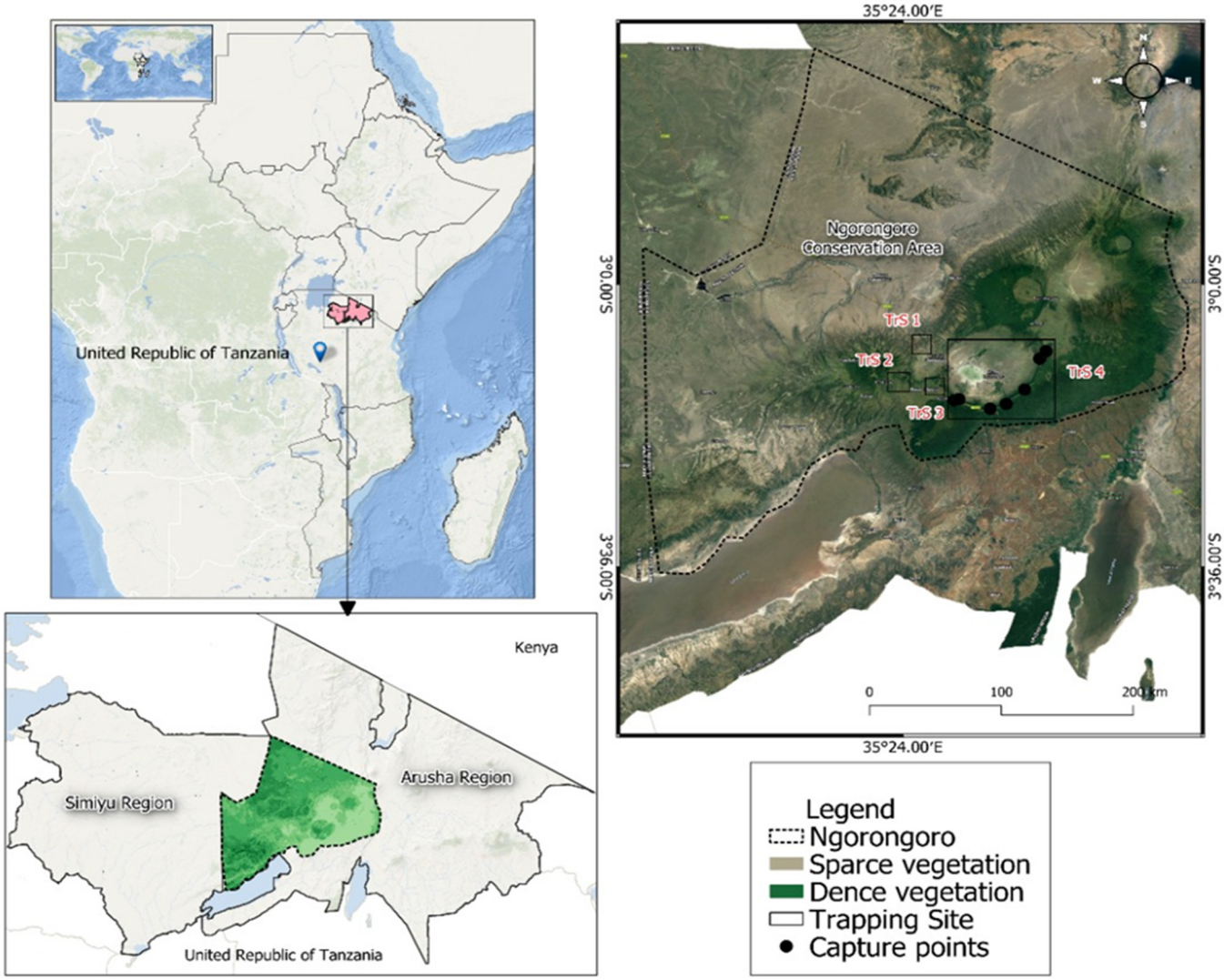

Figure 1. Distribution of *Cricetomys* spp. in Ngorongoro Conservation Area.

3.2. Detection of Leptospira Serovar

The detection of *Leptospira* in samples by MAT and PCR, and in cultures of urine and kidney tissues, varied broadly between the three techniques. Via MAT, 3 of the 50 samples (6%) tested positive (Table 2), of which 2 were positive for Lora (*L. interrorgans* serogroup Australis) and 1 for Sokoine (*L. kirschneri* serogroup Icterohaemorrhagiae). Via PCR, 10 out of the 50 (20%) kidney tissue samples were found to be positive for leptospiral genes (Figure 2). One sample showed positivity for either MAT or PCR. There was a significant difference between positive and negative cases between the methods (Fisher exact test, $p < 0.05$). No *Leptospira* serovar were detected via culturing from the kidney tissue or urine samples—5 culture samples out of 100 (5%) were contaminated and hence lost during the procedure.

Table 2. Antibody titers of rodents (n = 50) tested with MAT including six *Leptospira* serovars.

	Titer		
***Leptospira* serovar**	**1:20**	**1:40**	**1:160**
Sokoine	1	0	0
Lora	0	1	1
Hebdomadis	0	0	0
Canicola	0	0	0
Pomona	0	0	0

Figure 2. PCR product of DNA from kidney samples of Cricetomys spp. with Lepat 1 and Lepat 2 primers. LM = ladder marker; 1, 2, 4, 5, 8, 10 and 12 = positive samples; P.C = positive control; N.C = negative control; bp = base pair.

3.3. Distribution of Leptospira Serovars in Cricetomys spp.

The detection of *Leptospira* varied with the site of capture. All leptospira-positive *Cricetomys* spp. were captured near human settlements, including the tourist lodge (Table 3). Of the areas where *Leptospira* serovar were detected, three sites—Pongo, Kongoni, and Ngorongoro Wildlife Lodge—showed relatively high detections, while Viewpoint had one positive sample (Table 3). There was no significant difference in seroprevalence between the sites where *Leptospira* spp. was detected (Fisher exact test, $p = 0.57$). Sites that were littered with garbage had a higher seroprevalence of *Leptospira* spp. than the other sites (Table 3). Of the sites, Lerai was closest to a water pump, while the Lemala and Forest sites were close to streams. Both Lemala and Forest captures had no *Leptospira* detected in them, and had no dump sites in their vicinity.

Table 3. Trapping sites of African giant pouched rat in Ngorongoro Conservation Area.

SITE	Total Captured	Positive	% Positive	Presence/Absence of Dump Site	Presence/Absence of Water Body
NCA Wildlife lodge	6	3	6%	Present	Absent
Viewpoint	5	1	2%	Present	Absent
Pongo	11	3	6%	Present	Absent
Kongoni	14	3	6%	Present	Absent
Lerai	7	2	4%	Present	Present
Lemala	6	0	0%	Absent	Present
Forest	1	0	0%	Absent	Present
TOTAL	50	12	24%		

3.4. Influence of Season on Seroprevalence of Leptospira spp. in Cricetomys Rats in the NCA

There was no significant difference in the seroprevalence of *Leptospira* spp. in *Cricetomys* rats between the dry and wet seasons (Fisher exact test, $p = 0.18$).

4. Discussion

To our knowledge, this is the first study reporting on the seroprevalence of *Leptospira* spp. in *Cricetomys* spp. in the NCA. The study shows that *Cricetomys* spp. were prevalent in the forest vegetation in the NCA where there was adequate cover for these rats against predation. In addition, the relatively high humidity in this area is favorable for the survival of *Cricetomys* rats in terms of foraging and nesting burrows [38]. The forest is located in the highlands of the Ngorongoro Crater and receives relatively more rainfall than the other vegetated environments [49,50].

There was a variation in the results obtained using the different laboratory diagnostic tests. Via culturing, it was not possible to demonstrate live leptospires, indicating that culturing was not sensitive enough to detect *Leptospira* in the kidney tissues or urine samples, possibly due to contamination by faster-growing microorganisms, since leptospires are slow-growing microorganisms [51], often due to the long period of incubation required to achieve the growth of *Leptospira* [24,52]. Furthermore, this classical detection method does not take into account the existence of viable but uncultivatable organisms, which is evident in many other bacteria but not *Leptospira* [53–55].

MAT detected more *Leptospira* serovar antibodies in the serum than culturing from kidney and urine samples. This is different to the findings of other studies that showed that the culture method had a higher sensitivity than MAT [3,54] but comparatively lower sensitivity compared to PCR. The argument over which is the most reliable method for the diagnosis of leptospirosis is not a recent issue [56]. Nevertheless, MAT remains the gold standard for *Leptospira* diagnosis in epidemiological studies because it detects the presence of IgM and IgG antibodies. The replacement of MAT is difficult because it is cost-efficient and in addition offers an abundance of information [20].

Serovar Lora (Australis serogroup) showed a higher seroprevalence in the *Cricetomys* spp. than serovar Sokoine (Icterohaemorrhagiae serogroup), although previous studies have demonstrated serovar Lora to be more common in smaller rodents [13,14]. Serovar Sokoine was originally isolated in cattle; this is a contributing factor to its lower seroprevalence in *Cricetomys* spp. [57]. This implies that serovar Lora could be the most prevalent serovar circulating in *Cricetomys* spp. in the Ngorongoro Conservation Area.

The seroprevalence of *Leptospira* spp. in African giant pouched rats in the NCA was 24%, which is higher than the seroprevalence values of 8.4% and 20.29% reported elsewhere [13,14] in Tanzania. This might be due to climatic differences between the study areas [34], such as extended rainy periods and high humidity in the NCA compared to Morogoro and Katavi, where the other studies were conducted. Additionally, this study's sample size was relatively smaller than those in earlier studies conducted elsewhere in Tanzania [13,14].

In this study, the seroprevalence of *Leptospira* spp. was almost the same in each trapping site. However, there was some variations among the habitats sampled. For instance, sites closer to human settlements detected high seroprevalence compared to other sites. This is argued to be due to the poor management of refuse by humans, a situation that attracts rats including the nocturnal African giant pouched rat, and allows for high levels of interaction that lead to leptospira contamination in the environment. The contamination increases the probability of infection in other *Cricetomys* spp. that come to forage in the garbage sites [58]. According to other studies [59,60], infected rodents can remain carriers of leptospires for life. *Cricetomys* spp. individuals that were negative for *Leptospira* serovar (Table 2) were trapped in sites with no garbage dumps in their vicinity, although they were near water bodies (water pumps and streams). This suggests that the presence of garbage in the vicinity is a major factor that attracts rodents, including maintenance hosts of the

pathogen, which in turn contaminate the environment [53]. Moreover, these sites featured a wet environment, which by itself did not contribute to the presence of the leptospires.

The seroprevalence of *Leptospira* spp. was higher in the dry than in the wet season. This is striking because *Leptospira* serovar survive best in more moist and cool surroundings. However, the forest vegetation of the NCA is characterized by relatively low temperatures and high humidity during the dry season [35,50]. This implies that the environmental condition in NCA supports the survival of *Leptospira* and its maintenance host.

Implications and Limitations

Findings on the presence of the *Leptospira* bacteria circulating in the NCA have a significant implication for the local communities, staff, and tourists in the NCA. These bacteria can be transmitted to humans, wildlife, and livestock in the NCA. Leptospirosis diseases caused by the *Leptospira* bacteria can cause a decrease in productivity, and severe cases cause death in humans, wildlife and livestock.

Several limitations were met when conducting this research, including a small sample size because of the nature of the study area, which is a conservation area, and limited serovars were tested. We focused only on the prevalent serovars circulating in the region, as well as host species.

5. Conclusions

It is concluded that *Cricetomys* spp. are reservoirs of the *Leptospira* serovar in the NCA, and are more abundant in the vicinity of human settlements where diverse food attractants are available.

Residents and tourists in the NCA are, therefore, at risk of being infected upon coming into contact with water bodies, soils, and vegetation contaminated with urine of *Cricetomys* spp. Therefore, to avoid infection, environmental sanitation should be emphasized around human settlements, and awareness of leptospirosis and its transmission should be imparted to the NCA population by public health staff of the conservation area. Rodent control should be carried out strictly where necessary, and under the supervision of conservationists to minimize ecosystem disturbances in the NCA.

Common serogroups in *Cricetomys* spp. have been demonstrated in this study. There is a high probability of the *Leptospira* serovars detected spilling over into domestic and wild animals. The findings of the study also highlight the possibility of transmission from host species to humans due to their close proximity within the conservation area. Hence, the serovars identified in this study from the NCA can be used in the future in the MAT antigen panel to detect *Leptospira* serovars in the humans and domestic and wild animals that co-exist in the NCA.

We recommend that human activities in the NCA should be restricted, and conservation strategies should be promoted, since the circulation of *Leptospira* serovars is influenced by the presence of human settlements. Reduced interaction between humans and rodents is safer.

Further studies can be performed on other rodent species, wildlife, humans and livestock in the NCA to understand their role as maintenance and accidental hosts of *Leptospira* serovars.

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Informed Consent Statement: Not applicable.

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