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Serological similarity of Leptospira serovars in livestock and livestock keepers in Ngorongoro conservation area, Tanzania

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Abstract

Background: Leptospirosis is a bacterial zoonotic disease affecting mammals including wildlife, livestock, and humans, with higher prevalence recorded in pastoral communities where close contact between animals and humans increases the risk of transmission. This study aimed to determine the seroprevalence of Leptospira serovars among livestock and livestock keepers in four selected villages of the Ngorongoro Conservation Area (NCA), during the wet season.

Methods: A cross-sectional study was conducted from February to April 2022 in four villages within NCA. The convenience sampling technique was recruited to collect blood samples from 141 livestock (96 cattle, 25 sheep, and 20 goats) and 154 livestock keepers. Sera were analyzed using the Microscopic Agglutination Test (MAT) to diagnose individuals infected by Leptospira serovars. Data were analyzed using SPSS v26 with descriptive and inferential statistics including Chi-square, Kruskal-Wallis, and logistic regression.

Results: The overall seroprevalence was 10.64% in livestock and 5.19% in livestock keepers. Cattle had the highest positivity (11.46%) among animals. Hebdomadis was the most frequently detected serovar in livestock, while Grippotyphosa predominated in humans. Oloirobi village recorded the highest human seroprevalence (3.25%) with a statistically significant difference compared to Endulen (p=0.007). Analysis of logistic regression showed no significant associations between seropositivity and sex or age of livestock keepers.

Conclusion: The presence of Leptospira antibodies in both livestock and humans indicates ongoing exposure in the NCA. These findings underscore the need for targeted interventions including livestock vaccination programs, improved animal husbandry practices, and increased public health education for livestock keepers to reduce transmission risks and protect both animal and human health.

Keywords: Leptospirosis, Prevalence, Microscopic Agglutination Test, Seroprevalence, Livestock, Tanzania

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Background

Leptospirosis is a bacterial zoonotic disease affecting mammals including wildlife, livestock, and humans in tropical and sub-tropical regions of the world [1,2]. There are more than 300 described serovars of the Leptospira spp. all over the world [3]. In Tanzania, the commonly described serovars include serovars Sokoine, Kenya, Grippotyphosa, Pomona, Lora, and Hebdomads [2-6]. Infected animals harbor the bacteria in their kidney tubules for a long time and infect the environment with their urine [7]. Humans are incidental hosts and get infected by direct contact with the urine or, indirectly, through environmental materials like water and soil that have been contaminated with the urine or exudates from infected animals [8]. Many serovars of Leptospira spp. can infect humans, with some exhibiting host specificity; for example, serovar Sokoine isolated in Tanzania, which usually infects cattle and rodents [9-11], or serovar Kenya which primarily infects rodents and shrews. Serovar Lora infects rodents while Grippotyphosa and Pomona infect cattle and swine, respectively. Serovar Hardjo which is also known as bovine-adapted serovar, is also host-specific commonly infecting cattle [12-15]. Leptospirosis is not routinely diagnosed in healthcare facilities in Tanzania [11] and studies have shown that leptospirosis is an occupational disease affecting rice and sugarcane farmers, fish handlers, livestock keepers, and abattoir workers, among others [16]. Like malaria, typhoid, dengue, and other human febrile illnesses, symptoms of leptospirosis in humans consist of headache, vomiting, and muscle pains [1].

Consequently, leptospirosis is often mistaken for other common febrile illnesses [17-19]. When it occurs to livestock, livestock keepers suffer financial losses as a consequence of animal abortions, decreased fertility, decreased milk production, or decreased carcass weight of animals infected with leptospirosis [20]. Studies of leptospirosis in humans, domestic animals, and wildlife have been done in some regions of Tanzania [2,21,22]. However, there are limited studies in livestock-keeping communities where the probability of infection is high due to prolonged close interaction between humans and their livestock, which are one of the major hosts of the pathogen [23-25]. Ngorongoro Conservation Area (NCA) different from other conserved areas in Tanzania allows humans, wildlife, and livestock to interact freely, therefore, the chances of cross-infection by the Leptospira are high [26]. Since there was inadequate information on the transmission link between livestock and livestock keepers, this study aimed at understanding the burden of leptospirosis, the possibility of transmission, and prevalent serovars in livestock keepers and their livestock in NCA, Tanzania. The results of this study can help with future diagnosis of leptospirosis in NCA and develop interventions to inhibit the transmission of the disease.

Methods

Study Area

The study was conducted in NCA, Ngorongoro district, Arusha region. NCA as a multiple land use area allows wildlife, livestock, and human co-existence [27]. It is a traditional home to the Maasai pastoralists and their livestock [28]. It is situated between 34 to 36° E and 2°30' to 3°37' S [29] over a surface area of 8,283km2 (Figure 1).

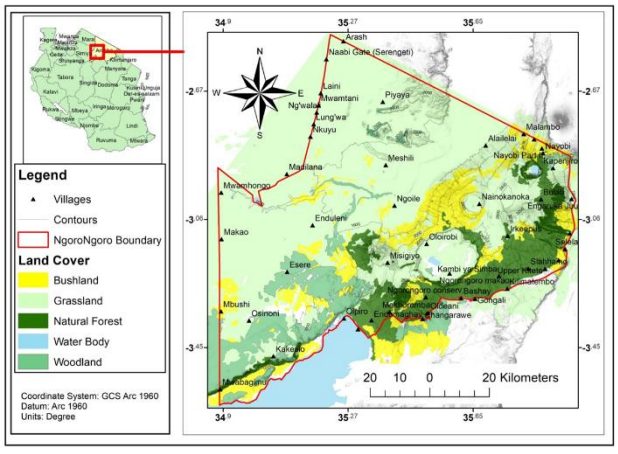

Figure 1: Map of Ngorongoro Conservation Area showing different villages.

Study design and sampling strategies

A cross-sectional study was conducted in the Ngorongoro Conservation Area in four selected pastoralist villages; Endulen, Oloirobi, Esere, and Irkeepus from February to April 2022 during the wet season. The villages for sampling livestock were selected using convenience sampling with the help of the Veterinary Department of Ngorongoro Conservation Area Authority (NCAA).

Inclusion and exclusion criteria

The inclusion criteria for Livestock keepers were, those aged 18 years and older, who live in NCA and are willing to participate in the study, patients seeking medical care from the local health facilities within the villages, who were not severely ill during the time of recruitment, with no history of leptospirosis case. Informed oral consent was provided before taking blood samples from livestock keepers. The exclusion criteria were non-livestock keepers, non-NCA residents, livestock keepers below 18 years, anyone severely ill during the collection of blood samples from study participants, also those who did not go to the hospital to get medical care, and finally individuals who did not agree to consent. The inclusion criteria for livestock were all livestock living in NCA within the four selected villages, whose owners provided informed oral consent for sample collection. The exclusion criteria for livestock were livestock outside of NCA, livestock outside the four selected villages, and livestock whose owners did not provide oral consent for sample collection.

Sample size

The sample size was determined based on the availability of livestock and livestock keepers in the study villages within the given time period. The study involved 141 livestock (96 cattle, 20 goats, and 25 sheep) and 154 livestock keepers that inhabit the NCA. Estimation of sample size for both livestock and livestock keepers used convenience sampling, whereby participants were enrolled in the study based on their willingness to participate [25].

Sample collection and handling procedure

Human blood samples (1mL each) were aseptically collected from each individual by human laboratory technologists from the brachial vein in 5mL plain vacutainer tubes using a 21-gauge needle. Before and after collection, the wound site was sanitized with gauze, previously soaked with an antiseptic to prevent infection [31]. Cattle, sheep, and goats were manually restrained, and blood samples were aseptically collected from the jugular veins using 10ml plain vacutainer tubes. The blood samples from animals and humans were allowed to clot in a slanted position, and serum samples were harvested after 24h [22]. The sera harvested from animals were dispensed into appropriately labeled 1.5ml cryovials and stored in liquid nitrogen (−78°C) to be transferred to the Institute of Pest Management laboratories, Sokoine University of Agriculture, and then kept in an ultra-deep freezer (−80°C) for MAT [22].

Microscopic Agglutination Test

Blood samples were collected from both livestock and livestock keepers residing in the selected villages for serum preparation. Antibody screening was carried out by the Microscopic Agglutination Test (MAT) [17,30]. The sera were examined for antibodies against Leptospira serovars by MAT, this is the serological gold standard assay for leptospirosis diagnosis [23]. Live leptospiral antigens of serovars Grippotyphosa, Lora, Hebdomadis, Sokoine, Pomona, Canicola, and Kenya were used. These serovars were chosen on the basis of previous research conducted to determine their prevalence in Tanzania's Northern region [2,24]. Serovar Hebdomadis and Grippotyphosa were isolated from cattle, previously, and Lora and Pomona were isolated from rodents, cattle, and buffaloes, respectively [12]. Domestic animals have also been identified to harbor serovars in Kenya and Canicola [9,25].

Growth of live antigens

The live antigens were cultivated in newly prepared Ellinghausen and McCullough medium, modified by Johnson and Harris (EMJH) (Difco-USA), with 5-fluorouracil as an antimicrobial additive and complemented with Leptospira enrichment until they attained a density of 300 × 108 leptospires/ml., after five to seven days of incubation at 30°C [2,32]. A loop-full of the stock culture was studied under a dark field (DF) microscope to confirm that there were no contaminants and that leptospires were viable (actively motile).

MAT procedure

In short, 10µl of serum samples was diluted using 90 µl of PBS in a U-shaped microtiter plate. Serial dilution was done by taking 50 µl of serum-PBS mixture from the tested row of each microtiter plate to the next rows containing 50 µl PBS consecutively that is 1;10, 1;20, 1;40, 1;80, 1;160, etc. Then after, 50 µl of antigen was added to all wells of the microtiter plate and thus doubled the dilution from 1;10 to 1;20, 1;20 to 1;40, 1;40 to 1;80, 1;80 to 1;160, etc. in the microtiter plate, etc. In each plate, the first upper row served as negative control; the test was made in the second row for each microtiter plate [33].

Data analysis

Data was deposited in Microsoft Office Excel® and analysis was done using IBM SPSS version 26 (2019) [34]. Descriptive analysis included frequency, median, and percent. Additionally, the inferential analysis included the Fisher exact test, Chi-square test, Kruskal Wallis test, and logistic regression, which was used to compare the prevalence of leptospirosis with a p-value ($p<0.05$) considered statistically significant [35].

Results

Demographic characteristics

The study involved 141 livestock from the Ngorongoro Conservation Area. Livestock consisted of cattle (96), sheep (25), and goats (20) (Table 1).

Prevalence of Leptospira serovars in livestock

The prevalence of Leptospira serovars in the livestock from four villages is shown in Table 2; Endulen (2.13%), Oloirobi (2.84%), Irkeepus (3.55%), and Esere (2.13%) were compared using Kruskal Wallis test and found no significant difference between the villages ($\chi2=6.10$, df=3, p=0.895) although Irkeepus village had the highest prevalence (Table 2).

Table 1: Seroprevalence by MAT in livestock from NCA

Livestock	Number of samples	Positive samples	Individual prevalence	Total prevalence
Cattle	96	11	11.46	7.14
Sheep	20	2	10	1.3
Goat	25	2	8	1.3

Table 2: Comparison of Leptospira seroposivity in Livestock in selected villages

Number of Livestock				Seropositivity				χ^2	P-value
Endulen	Oloirobi	Irkeepus	Esere	Endulen	Oloirobi	Irkeepus	Esere		
39	37	43	22	2.13% (3/141)	2.84% (4/141)	3.55% (5/141)	2.13% (3/141	6.10	0.895

Leptospira seropositivity in cattle, sheep and goats

Individually, the prevalence of serovars in cattle was 11.46% (11/96), sheep 8% (2/25), and goats 10% (2/20). Compared between cattle and sheep, cattle and goat, sheep and goats by chi-square test showed no significant difference in prevalence $p\geq0.05$. However, the total prevalence was higher in cattle than in the other livestock (Table 1). Results showed that the overall prevalence of leptospirosis in cattle was higher than in the other animals. A total of 15 (about 10.64%) of the samples in livestock had a positive result for at least one serovar at a cutoff point of 1:160. Hebdomadis showed the highest prevalence of 7.09% (10 positive cases), followed by Sokoine and Lora, each at 1.42% (two positive cases each), and Pomona and Grippotyphosa at 0.71% (one positive case each).

Table 3: Demographic characteristics of livestock keepers in NCA

Participants Characteristics	Frequency/Median	Percent/Interquartile range	Number of seropositive	Prevalence
Age	30	18-94		
Male	63	59.1%	1/154	0.65%
Female	91	40.9%	7/154	4.55%

Livestock keepers

The study involved 154 livestock keepers from NCA. The median age of livestock keepers was 30, the percentage of females being higher than males. Higher seroprevalence has been observed in female livestock keepers than males (Table 3). According to Table 4, different age groups showed different levels of prevalence. The highest prevalence (3.25%) is observed in the first group with the age range of 18-25. Out of 154 individuals tested, 8 were positive for at least one serovar, giving an overall prevalence of 5.19%. The prevalence varies by serovar with Grippotyphosa showing the highest prevalence at 3.25% (five positive cases) followed by Hebdomadis with a prevalence of 1.3% (two positive cases), Pomona and Lora each with a prevalence of 0.65% (one positive case each), serovars Canicola, Kenya, and Sokoine did not show seropositivity. Single livestock was positive for both Pomona and Grippotyphosa serovars, and one livestock keeper showed a co-infection with both Pomona and Grippotyphosa. Serovar Canicola and Kenya were neither detected in livestock nor in livestock keepers (Figure 2).

MAT titer between livestock keepers and livestock

The MAT titer levels were higher in livestock than in livestock keepers, as shown in Figure 3. This suggests the prevalence of leptospirosis is higher in livestock than in livestock keepers

based on the titer of 1:160 and above. Among the four selected villages, i.e. Enduleni, Oloirobi, Esere, and Irkeepus; there was higher human seroprevalence in Oloirobi 3.25% (5/154) than in Endulen 1.9% (3/154). There was no serovar detected in Esere, and Irkeepus; however, the number of participants in Oloirobi (30) was lower than that of Endulen (120). A statistically significant difference was observed between the seroprevalence in Oloirobi and in Endulen village ($\chi 2=10.018$, df=1, p=0.007).

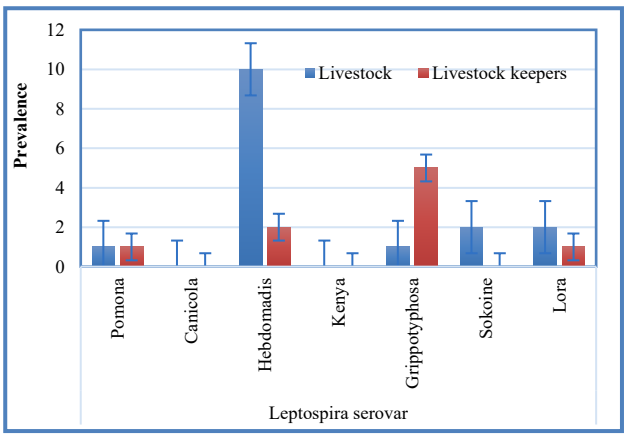

Figure 2: comparison of serovar prevalence between livestock and livestock keeper

Logistic regression analysis

Logistic regression was performed (Table 5) to predict the influence of age and sex on seropositivity in livestock keepers. There was no significant difference in seroprevalence between males and females, also there was no significant difference in seroprevalence in the different age groups (P≥0.05).

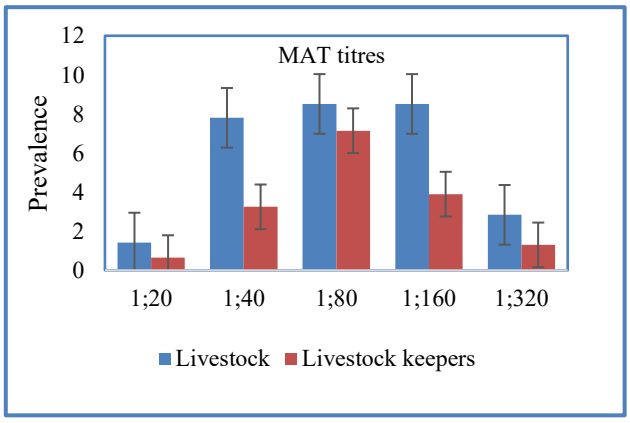

Figure 3. Comparison of MAT titre between livestock keepers and livestock

Discussion

This study diagnosed leptospirosis in the pastoral community in NCA. The findings show that leptospirosis is a public health threat to livestock keepers and livestock in the study area. The most common serovars were Hebdomadis and Grippotyphosa, in both livestock and livestock keepers [12,36]. Additionally, nearly all livestock keepers with higher seropositivity, also, had livestock with higher seropositivity in their village, this includes Endulen and Oloirobi with the exemption of Irkeepus [37,38]. Several studies have shown that domestic animals, particularly livestock such as cattle and pigs, can act as reservoirs for Leptospira spp. and are the key sources of transmission to humans, especially those working closely with animals. Research has proven that farm workers and individuals practicing animal husbandry are at a high risk of contracting leptospirosis due to continued exposure to infected animals or contaminated environments. This risk is worsened in environments where animals shed the bacteria through urine, which can then contaminate soil, water, and other surfaces, providing a channel to human exposure [39-42]. Serovar Grippotyphosa, belonging to Leptospira kirschneri, was among the most common serovars in livestock in the NCA, and it was also the most common serovar among livestock keepers. These findings concur with findings from another research conducted in the Kilimanjaro region in Northern Tanzania, which revealed Leptospira kirschneri as among the most prevalent Leptospira species in cattle, sheep, and goats [10]. This indicates that the more prevalent serovar could also be the most predominant serovar in the Ngorongoro Conservation Area. Irkeepus village had a high serovar prevalence in livestock but not livestock keepers; the reason could be due to a limited sample size of humans tested. Likewise, the low prevalence in Esere village was influenced by factors such as sample size limitation and reduced exposure to infected animals. This study has identified that the age range more susceptible to Leptospira infection is 18-25. This age range involves individuals who are engaged in many occupational activities that are a risk factor for contamination of Leptospira bacteria. Such activities are farming and animal husbandry that can lead to contact with contaminated soil, water, or infected animal's urine. This finding is supported by other studies showing that the risk of infection of leptospirosis increases with age due to occupational activities [43,44]. The study shows a higher seropositivity in female livestock keepers than in males, which differs from other studies that show males to have higher seropositivity than females [16,45]. Nevertheless, a study done in Endulen previously found high seropositivity in females, which agrees with our findings [46]. Higher seropositivity in female participants indicates that females in the pastoral community are at a higher risk because activities such as milking, feeding calves, and cleaning barns involve them being in closer proximity and contact with livestock [47], and their excreta directly [25,23,48]. The prevalence of leptospirosis in livestock keepers in NCA (5.19%) that this research reported was lower than that shown by a previous study in the pastoral community in Endulen, of 7.9%, in 2017 [49]. Common serovars detected in 2017 were Icterohaemorrhagiae, Djasiman, and Australis, while common serovars detected in this research were Grippotyphosa and Hebdomadis. The continuing decline in the population of livestock keepers in NCA could be the contributing factor to the lower prevalence of leptospirosis [25]. An additional factor is the change in climatic conditions over the years, whereby there has been a notable rise in temperature and altered rainfall patterns that could have impacted leptospirosis transmission among livestock keepers. Drier conditions due to temperatures increasing above average and less precipitation, make Leptospira spp. Less likely to survive in the environment and consequently lower infection rates. [50,51]. Another aspect is the increased antimicrobial treatment that may have been administered in both livestock and livestock keepers, which in recent has been on the rise for curing other bacterial infections [52]. The use of MAT, although cost-effective, could be an additional contribution to the low detection of Leptospira serovars in NCA because MAT was limited to the detection of only the serovars present in the antigen panel.

Table 4: Age range and prevalence in livestock keepers of NCA

Age group	Number of Seropositive	Prevalence
18-25	5	3.25%
26-35	1	0.65%
36-45	0	0
46-55	1	0.65%
56-65	0	0
66-75	1	0.65%
76-85	0	0
86-95	0	0

The use of modern techniques like PCR and whole genome sequencing could have given more confirmatory findings. Also, the sampling of individuals attending the health facilities, alone, could not necessarily be representative of the NCA livestock keeper population as there could be infected individuals who might not necessarily go to the health care facilities seeking treatments during the time of the study. Oloirobi and Endulen villages showed a higher prevalence of leptospirosis compared to Esere. Oloirobi and Endulen are in the lowlands, nearer to the Ngorongoro crater, compared to other villages that are up on the Ngorongoro highlands [53]. According to a study done in Tanzania [21], semi-arid areas usually have higher seropositivity than cooler temperate areas, and this is in agreement with the findings of our study. There is more frequent interaction between humans and animals nearer the crater area than in the highland areas of Ngorongoro. According to a study done by NCA, 82.1% of the waterholes are shared by humans, livestock, and wildlife making them the main source of disease transmission [54]. Leptospira seroprevalence was highest in cattle compared to sheep and goats. Previous studies in Tanzania have found that Leptospira spp may live in cattle, sheep, and goats, with differing prevalence rates. According to a study conducted in Northern Tanzania, pathogenic leptospiral infection was diagnosed in 7.08% of cattle, 1.12% of sheep, and 1.20% of goats sampled from nearby abattoirs [10,55]. However, studies conducted in Kilwa District discovered lower seroprevalence in cattle (26%), than in goats (27.5%), and sheep (30%) [56]. Generally, cattle have been described to be more susceptible to leptospiral infection because of their mating frequency [57]. There is higher Leptospira seropositivity in semi-arid areas of Tanzania than in humid areas because of the intensity of animal and human contact [21]. The variation in prevalence observed among several regions and different livestock species indicates that elements like the local setting, farming methods, different circulating serovars, and regional disparities significantly influence leptospirosis infection rates in cattle, goats, and sheep in Tanzania. Furthermore, the presence of rodents in the area increases the rate of infection of leptospirosis [26,58,59]. Sheep and goats exhibit different feeding behaviors, primarily sheep are grazers and goats are browsers. Their feeding behaviors influence contact with environmental pathogens such as leptospires. These livestock are well documented as reservoirs of several bacterial zoonoses, such as brucellosis, which exhibit some clinical signs similar to leptospirosis [60]. These livestock act as amplifiers of certain zoonotic pathogens from wild animals and play an important role in the spread of several diseases to humans [61]. The limitations of the study were inadequate funds to enable the use of molecular technologies which are more efficient in diagnosis and the unwillingness of livestock keepers to participate in the study which limited the sample size.

Conclusion

This study's results show that the prevalence of leptospirosis in NCA was generally low in both livestock and livestock keepers. Also, there is a significant difference in prevalence between Endulen and Oloirobi. The high seropositivity in both livestock and livestock keepers in two villages implies that livestock could be a possible source of transmission in those villages. In this case, livestock vaccination in NCA is necessary using identified local prevalent serovars as antigens in villages with higher seroprevalence. There is a need to raise awareness of leptospirosis among communities in NCA. Also, it is important that leptospirosis is included among the differential diagnostic tests in the health providing centers, in NCA, or at least in the hospitals. Furthermore, research using molecular technologies, such as Polymerase Chain Reaction (PCR), Next Generation Sequencing (NGS), and Nano biosensors can be used to discover new serovars in wildlife, humans, and domestic animals in NCA.

Abbreviation

NCA: Ngorongoro Conservation Area; MAT: Microscopic Agglutination Test; NGS: Next Generation Sequencing; PCR: Polymerase Chain Reaction.

Declaration

Acknowledgment

We appreciate the members of the Ngorongoro District local government for their support throughout the data collection period. We thank the staff at Ngorongoro Conservation Area (NCA) headed by Dr. Dickson Wambura for their assistance during data collection from livestock. We also thank all the medical staff at the health centers in Endulen, Oloirobi, Esere, and Irkeepus for their assistance during data collection from human participants from the pastoral community in NCA. The funds for the research were provided through the African Centre of Excellence for Innovative Rodent Pest Management and Bio-sensor Technology Development ((ACE IRPM and BTD)5799-TZ).

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Availability of data and materials

Data will be available by emailing priscamarilyn@gmail.com

Authors' contributions

The principal investigator (PI) of the research was Prisca N. Kahangwa (PNK). The research supervisors were Amani S. Kitegile (ASK) and Abdul S. Katakweba (ASK). Data analysis, interpretation, and article writing were done by Prisca N. Kahangwa with the assistance of Ginethon Mhamphi (GM) and Claus Thomas (CT). Article reviewing was done by Robert S. Machang'u (RM).

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical clearance for conducting this study was granted by the Institutional Review Board of Sokoine University of Agriculture (SUA/ADM/R.1/8/571, dated 17 January 2020). Ethical clearance to conduct medical study was granted by the National Institute for Medical Research (NIMR) (NIMR/HQ/R.8c/Vol. I/1941 dated 10th January 2022).

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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