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Sero-molecular epidemiological analysis of leptospirosis in smallholder dairy cattle in selected regions of Tanzania

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SERO-MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF LEPTOSPIROSIS IN SMALLHOLDER DAIRY CATTLE IN SELECTED REGIONS OF TANZANIA

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A Thesis submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The smallholder dairy industry in Tanzania is a promising sector for household income generation despite challenges of zoonosis such as leptospirosis caused by *Leptospira* serovar Hardjo. A total of 2086 blood, serum and additional vaginal swab for female cattle were collected from smallholder dairy cattle for leptospirosis testing using three complementary tests An overall prevalence of 13%, 13.1% and 13.7% for *Leptospira* serovar Hardjo by ELISA test, RT-PCR for pathogenic *Leptospira* spp. and MAT respectively. Based on ELISA test, the highest prevalence shown in Iringa 30.2% (95% CI 25.1 - 35.7%) and Tanga 18.9% (95% CI 15.7 - 22.6). In multivariable analysis, factors that were a significant risk in smallholder dairy cattle are: animals over 5 years of age OR = 1.41 (95% CI 1.05 - 1.90); indigenous breed OR = 2.78 (95% CI 1.47 - 5.26); hiring or keeping a bull for breeding purposes OR = 1.91 (95% CI 1.34 - 2.71); distance between farms of more than 100 meters OR = 1.75 (95% CI 1.16 - 2.64); livestock kept extensively (OR = 2.31, 95% CI 1.36 - 3.91); farms without cat for rodent control (OR = 1.87, 95% CI 1.16 - 3.02); farmers with livestock training OR = 1.62 (95% CI 1.15 - 2.27); temperature (OR = 1.63, 95% CI 1.18 - 2.26), and the interaction of higher temperature and precipitation (OR = 1.5, 95%CI 1.12 - 2.01). This findings provide an opportunity for future study to include a broad serogroups panel for more identification of common serogroups circulating in cattle for vaccine target in Tanzanian cattle population.
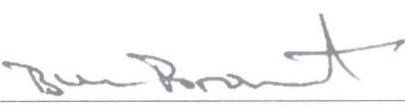
DECLARATION

I, **Shabani Kiyabo Motto**, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a thesis titled "Sero-molecular epidemiological analysis of leptospirosis in smallholder dairy cattle in selected regions of Tanzania" in partial fulfilment of the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This thesis is for my family and many friends who have been either directly or indirectly involved in this process. I owe a special debt of appreciation to my beloved mother, Hadija Masanja, for her prayers and words of support that pushed me forward in this endeavor. My lovely wife Mariam Mbwana deserves special recognition for her prayers and patience. She stood firmly caring my sons Irfaan and Irfad for the whole period when I was away from home. I also dedicate this thesis to my numerous friends, without mentioning everyone, who has helped me in many ways throughout the process. I will always be grateful for what they have done for me.

I am expressing my heartfelt gratitude to my best sister Tatu Kiyabo Motto and my brilliant brother Jumanne Kiyabo Motto for their unwavering support during my master's career. You have both been my biggest supporters.

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LIST OF ABBREVIATIONS

µL	Microliter
ACC	Arusha City Council
ADGG	African Dairy Genetics Gains
ARDC	Arusha Rural District Council
CAAT	Cross adsorption agglutination test
CTLGH	Centre for Tropical Livestock Genetics and Health
DALY	Disability Adjusted Life Years
DNA	Deoxyribose nucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzymes Linked Immunosorbent assay
gDNA	Genomic Deoxyribose Nucleic Acid
HDC	Hai District Council
HH	Household
HHH	Head of household
ID	Identification
IDC	Iringa District Council
ILRI	International Livestock Research Institute
IMC	Iringa Municipal Council
KCRI	Kilimanjaro Christian Research Institute
KRDC	Korogwe Rural District
KUDC	Korogwe Urban District Council
LDC	Lushoto District Council
LGAs	Local government authorities
LiSBE	Life Science and Bio-Engineering
LPS	Lipopolysaccharide

MaDC	Makambako District Council
MAT	Microscopic agglutination test
MaTC	Makambako Town Coucil
MbDC	Mbozi District Council
MCC	Mbeya City Council
MDC	Mbeya District Council
MeDC	Meru District Council
MoRDC	Moshi Rural District Council
MTC	Mafinga Town Council
MuDC	Mufindi District Council
MuDC	Muheza District
NC	Negative control
NDC	Njombe District Council
NEC	Negative extraction control
NM-AIST	Nelson Mandela African Institution of Science and Technology
NTC	Negative template control
NTC	Njombe Town Council
NTD	Neglected tropical diseases
OD	Optical density
ODK	Open Data Kit
PR	Positivity ratio
PRAs	Participatory rural appraisal
PTC	Positive template control
qPCR	Quantitative/Real-time polymerase chain reaction
RoDC	Rombo District Council
RPM	Revolution per minutes
RuDC	Rungwe District Council

SDC   Siha District Council

SHD   Smallholder dairy

SHDF   Smallholder dairy farmers

SHZ-X   Shorthorn zebu cross

TCC   Tanga City Council

TMB   Chromogenic substrate

TVLA   Tanzania Veterinary Laboratory Agency

VVBD

      Vector and Vector-borne Disease

CHAPTER ONE

INTRODUCTION

1.1   Background of the Problem

Leptospirosis is an important bacterial infection not only because of its health and production detrimental effects on cattle but it can be transmissible to people as well (Doungchawee *et al.,* 2013; Wasiński & Dutkiewicz, 2013). Many leptospirosis incidences are often reported in tropical and subtropical countries as they receive enough rain and moderate temperature that favour leptospires survival in the environment for a few weeks to months before picked by host (Allan *et al.,* 2018; Biggs *et al.,* 2013; Mgode *et al.,* 2014). Leptospirosis causes a adverse health effects in people (Ghasemian *et al.,* 2020). More than 250 pathogenic serovars that are collectively grouped into 25 serogroups based on their antigenic dissimilarities in the genus *Leptospira* can cause disease in people as well as a range of animal species (Cilia *et al.,* 2020; Kallel *et al.,* 2020; Lauretti-Ferreira *et al.,* 2020). Many serovars are known to cause reproductive disorders in cattle (infertility, abortion, and stillbirths), decreased milk output, increased treatment expenses for farmers, and economic loses (Alinaitwe *et al.,* 2020; Nthiwa *et al.,* 2019). However, may perhaps be subclinical symptoms presented by some serovars such as Sejroe, Hardjo, and Pomona at a time (Desvars *et al.,* 2013; State *et al.,* 2012; Waitkins, 2008) thus, increasing disease chances of perpetuation and transmission via human-animal contact. The presence of natural animal carriers such as rats in a human settlement, particularly in the dairy farming system, enhances the likelihood of leptospirosis infection and transmission (Ricardo *et al.,* 2020).

Leptospirosis is transmitted either indirectly via contaminated pastures, water sources, or between animals during grazing (De-Brito *et al.,* 2018). Pathogens are normally shed in urine and indirectly contaminate pastures and water sources or through milk, aborted fetuses, and/or placental fluid of infected animals (Benavidez *et al.,* 2019; Trevejo *et al.,* 1998). Uninfected animals acquire infection indirectly via contaminated pastures and water sources, while humans with abraded or skin cuts can disease contract when handling animal feces, milking, drinking unpasteurized milk, or milk plash during milking practice (Ribeiro *et al.,* 2017; Schneider *et al.,* 2013). Bacteria can also enter the body through organs of the soft-lined membrane such as the eye, mouth, or wounded skin, and some serovars can enter via the venereal (Nally *et al.,* 2020; Tekemen *et al.,* 2020). The most occupational risk group in the

dairy industry are milkers, veterinarians, butchers, and slaughterhouse workers who can easily contract pathogens as some serovars are maintained in cattle urinary tract (Schelotto *et al.,* 2012).

A quite number of studies and some carried out for many past years demonstrating leptospirosis in Tanzania. The first case of leptospirosis in cattle was reported in the late 1990s (Machang'u *et al.,* 1997). Later 21.3% of cattle shedding *Leptospira* pathogen in urine were reported in East Usambara Mountains, Tanga region (Karimuribo *et al.,* 2008). Also, 15.1% of human leptospirosis has been documeneted in Tanga region (Schoonman *et al.,* 2009). This is comparable to previous studies conducted on human and wildlife interfaces in the Katavi region that informed 30.37% leptospiral seropositivity in indigenous cattle, 28.95% in buffaloes, 29.96% in humans, 20.29% in rodents, and 9.09% in shrews (Assenga *et al.,* 2015). Similarly, study in the northern part of Tanzania described human acute leptospirosis in a hospitalized patients with the majority associated with livestock activities (Biggs *et al.,* 2013). *Leptospira* serovar Hardjo is the manjor cause of abortion and sharp drop milk production in dairy industry. Despite the importance and growth of dairy industry in Tanzania, seroprevalence of *Leptospira* serovar and other serovar is not clear known in economically important dairy regions. Smallholder farming system remained isolated and less informed on occurrence of pathogenic *Leptospira* that may causes economic losses in smallholder dairy farmers in Tanzania. The present study was carried out to determine prevalence of *Leptospira* serovar Hardjo and additional important *Leptospira* serovars in Tanzanian dairy cattle for better management of the disease.

1.2   Statement of the Problem

Despite the potentiality of the dairy industry of its promising sector for household income contribution, creating employment, and improving livelihood and food security for smallholder farmers, leptospirosis remained a challenge in livestock with previous studies evidence. Many studies mentioned leptospirosis seropositivity in many species including humans, animals, and wildlife (Assenga *et al.,* 2015; Ngugi *et al.,* 2019; Schoonman *et al.,* 2009). In livestock, mainly *Leptospira* serovar Hardjo causes a serious abortion, calf deaths, lower milk output, expenditures together with animal treatment, and increased running costs for a cure when workers become ill. These are all examples of financial losses in the dairy sector caused by the disease (Ijaz *et al.,* 2020; Wasiński *et al.,* 2012). However, leptospirosis may show asymptomatic cases can be seen in cattle and pigs for the infecting *Leptospira* spp. serovars

Hardjo and *Leptospira* spp. serovars Pomona respectively (Cook *et al.,* 2017). Leptospires can persist in the urinary and genital tracts for a long period with no clinical symptoms while shedding via urine (Calderón *et al.,* 2014). Maintaining the pathogen in the urinary and genital tract with no clinical symptom accelerates the spread of the disease particularly when introducing new animals into the herds without screening and in co-grazing practice in pastoralists (Acestor *et al.,* 2012).

The impact of leptospirosis is not only significant in livestock but also in spreading serovars to other accidental hosts in the same habitation including livestock farmers especially when the herd follow in asymptomatic infection (Lilenbaum & Santos, 1996). Though, vaccination programs are promising and feasible strategies to control leptospirosis animals by preventing spread and disease outbreak (Okosun *et al.,* 2016), it essential to establish the serovars panel that circulates in a specific geographical area for appropriate vaccine use (Goarant, 2016).

Recently, status of *Leptospira* serovar Hardjo in smallholder dairy cattle is scarce in Tanzania coupled with neither a necessary control program nor active surveillance in place. In combination of unknown status of leptospirosis in dairy cattle increases the probability of farmers contracting the disease (Maziku *et al.,* 2017). The smallholder dairy (SHD) industry is one of Tanzania's fastest-growing industries, accounting for 30% of the entire livestock sector (Njombe *et al.,* 2011) and serves 80% of Tanzanians living in rural and peri-urban areas, with more than 90% of smallholder livestock keepers relying on hand milking procedures. The growth of the SHD business in Tanzania, particularly in rural and peri-urban regions, is accompanied by inadequate environmental sanitation that poses smallholder dairy farmers (SHDF) at risk of zoonoses such as leptospirosis (Ribeiro *et al.,* 2017).

1.3   Rationale of the Study

The study helped to establish risk factors and a baseline of *Leptospira* serovar Hardjo in smallholder dairy farmers. The study also highlited the hotspot area of leptospirosis in dairy cattle which will help policymakers and stakeholders as point of start when it come on disease control strategies especially in Iringa, Njombe from the southern and Tanga from the northern part of Tanzania. This study also inform that most of the community need awereness in regards of leptospirosis and other zoonosis as well. Furthermore, this study opened a chance for future to focus on other side investigating leptospirosis in human and transmission dynamic of the disease from livestock to human.

1.4   Research Objectives

1.4.1   General Objective

The overall goal of this research was to understand the prevalence of *Leptospira* serovar Hardjo as the major couse of economic loses in dairy cattle, and it spatial distribution for effective disease control.

1.4.2   Specific Objectives

The study aimed to achieve the following specific objectives:

(i)   To determine seroprevalence, the spatial distribution of *Leptospira* spp. serovars Hardjo that cause economical loses in dairy cattle and it associated risk factors in selected regions of Tanzania.

(ii)   To identify other *Leptospira* serogroups (Hebdomidis, Sokoine, Lora, Grippotyphosa and Pomona) in smallholder dairy cattle populations in selected regions of Tanzania based on microscopic agglutination test (MAT).

(iii)   To evaluate the presence of pathogenic *Leptospira* spp. using real-time polymerase chain reaction targeting the *lip*L32 gene.

1.5   Research Questions

The study intended to answer the following questions:

(i)   What are the prevalence, risk factors and spatial distribution of *Leptospira* serovar Hardjo in smallholder dairy cattle in Tanzania selected regions?

(ii)   What are additional serogroups of *Leptospira* spp based on the microscopic agglutination test are circulating in dairy cattle in Tanzania?

(iii)   Is there pathogenic *Leptospira* spp encoded with *lip*L32 gene circulating and causing disease in Tanzanian smallholder dairy cattle?

1.6   Significance of the Study

Despite numerous studies stated the impact of leptospirosis in dairy industry in particular *Leptospira* serovar Hardjo, dairy farming in Tanzania remained a promising sector for house hold income generation. Dairy farming system in Tanzania serves over 30% of the entire livestock sector however, the sector remained isolated and less informed on occurrence of pathogenic *Leptospira* serovar Harjo that may causes economic losses in smallholder dairy farmers in Tanzania and infection to human.

In this regard, the study gives a comprehensive exploration of *Leptospira* serovar Hardjo in smallholder dairy cattle in Tanzania. Limited awareness to dairy farmers on leptospirosis might affect transmission dynamic of the disease from animal to people, therefore this study inform the need of leptospirosis and zoonosis awareness raising to dairy farmers as a control strategies of the disease in farms. Moreover, through the findings and disease mapping made in this study, policy makers and stake holder in dairy industry will have an opportunity to prioritize area to work on to control disease.

In addition, the overview presented in this research will push for new paradigms, which will be helpful for future study to integrate the leptospirosis in human, environmental factors and other reservoirs including rodents.

1.7   Delineation of the Study

This study explored leptospirosis in smallholder dairy cattle in Tanzania by using various diagnostic approach. Despite the microscopic agglutination test (MAT) being a gold standard test for leptospirosis, this study deployed only five serovar panel out of over 200 serovar that are known to cause disease in cattle. Furthermore MAT does not distinguish between the current or previous infection. Since the disease is the zoonotic that can spread to human, the estimation of does not reflect on the status of the disease in human especially dairy farmer if there any disease interaction between animals and farmers.

CHAPTER TWO

LITTERATURE REVIEW

2.1   Structure and Biology of Leptospires

Leptospires are bacterial agents capable to cause a disease called leptospirosis in a wide range of hosts from animals, humans, wild and aquatic life (Benacer *et al.,* 2017; Dib *et al.,* 2014; Tique *et al.,* 2018). The bacteria are flexible, motile, and tightly coiled at one end, forming a question mark shape. The structure can easily be distinguished from other spiral organisms Figure 1 however, leptospiral serovars can only be differentiated by either serological or molecular techniques (OMS, 2003).

The cytoplasmic membrane and cell wall make up the structure of leptospires. They are gram-negative bacteria because their cell walls contain peptidoglycan, which makes them resistant to gram stain (Adler & De-la-Peña-Moctezuma, 2010). Because organisms are very tiny, therefore can only be viewed under a dark field, phase-contrast microscope or electronic microscopic. The size of bacteria is typically between 0.1- 0.2µm in diameter and 6 – 20 µm in length (Goarant, 2016; Schreier *et al.,* 2013).
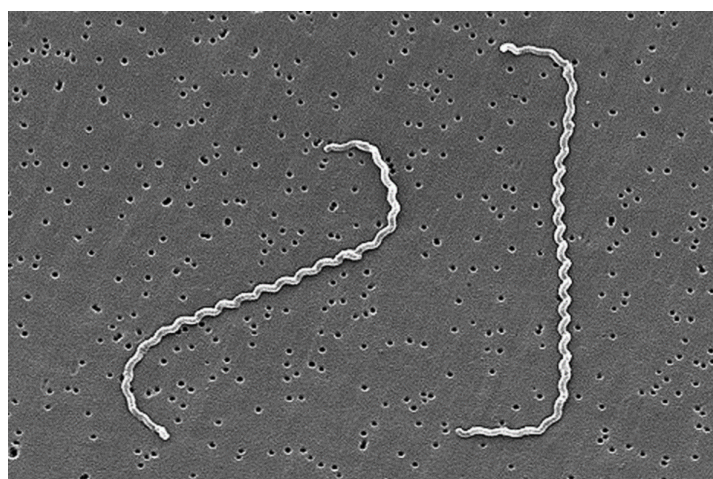
Figure 1:   **High resolution of *Leptospira* interrogans serovar icterohaemorrhagiae under a scanning electron microscope (Levett, 2001)**

Leptospires are obligate aerobes bacteria that require oxygen to survive at temperatures ranging from 28°C to 30°C and a pH of 7.8 (Mohammed *et al.,* 2011; Schreier *et al.,* 2013). Bacteria can also grow in vitro (in a controlled environment outside of the host), however additional nutrients, such as fatty acids to function as a source of energy are highly required (Biggs *et al.,* 2013; Page, 2013).

2.2   Classification of Leptospires

Classically, leptospires are grouped in the genus *Leptospira*, family Leptospiraceae and phylum Spirochetes (Mohammed *et al.,* 2011). They contain a unique arrangement of lipopolysaccharides (LPS) on the outer surface which is a fundamental function for classification from serovar to serovar with serological techniques (Vijayachari *et al.,* 2015). Additional LPS epitopes on their outer surface are responsible for virulence determinants (Lauretti-Ferreira *et al.,* 2020).

In 1989, the cross adsorption agglutination test (CAAT) was used to classify serovars in the genus *Leptospira* (Backstedt *et al.,* 2015). Genus *Leptospira* was separated previously into pathogenic *L. interrogans* and non-pathogenic (saprophytic) *L. biflexa.* Each species was further subdivided into numerous serovar (Levett, 2001). Currently, the genus *Leptospira* has more than 20 serogroups divided into not less than 250 serovars. Some of these serovars are known to cause sickness in people and a variety of animal species (Ngugi *et al.,* 2019; Surujballi & Mallory, 2004).

With the discovery of new sophisticated techniques and the emergence of various serovars, *Leptospira* is recently classified based on DNA homology. The genus *Leptospira* is divided into three lineages (Allan *et al.,* 2016) of both veterinary and medicinal significance (Chiriboga *et al.,* 2015; Vincent *et al.,* 2019); which are pathogenic, intermediate, and non-pathogenic of 20 species (Allan *et al.,* 2016; Goldman & Schafer, 2012) (Table 1).

Table 1:   Classification of *Leptospira* spp. based on their pathogenicity lineage

Pathogenic	Intermediate	Non-pathogenic
L. interrogans	*L. wolffii*	*L. biflexa.*
L. kirschneri	*L. inadai*	*L. meyeri.*
L. noguchii	*L. fanei*	*L. terpstrae.*
L. weilii	*L. broomii*	*L. vanthielii.*
L. borgspetersenii	*L. licerasiae*	*L. wolbachii.*
L. santarosai		*L. yanagawae.*
L. alexanderi		
L. alstonii		
L. kmetyi		

The newest update showed *Leptospira* classified into 64 species collectively grouped into two clades (pathogenic "P" and saprophytic "S") and two subclades in each clade (subclades P1 and P2; and subclades S1 and S2, respectively). The clade P, subclade P1 and P2; 17 and 21 species respectively, and the 6 non-pathogenic placed in clade S subclade S1 (Vincent *et al.,* 2019).

2.3   Epidemiology and Burden of Leptospirosis

Leptospirosis is the re-emerging infectious and notifiable zoonosis in many developing countries (Doungchawee *et al.,* 2013; Sharma *et al.,* 2019). It is widely documented globally (Doungchawee *et al.,* 2013; Wasiński *et al.,* 2013), and often occurs in both tropics and subtropics countries (Fig. 2). Leptospires can survive for a week to several months in humid to relatively warm environments (Krijger *et al.,* 2019; Fernandez *et al.,* 2020), and in swamps (Jittimanee & Wongbutdee, 2019). The emergence of disease and health impacts are largely proportion upsurges in developing countries (DCs) where repeatedly surveillance and control strategies are poorly achieved (Rajapakse *et al.,* 2015; Yadeta *et al.,* 2016). Furthermore, urbanization linked to slum settlement of poor environmental hygiene, substantial rains, and flooding accelerates the spreading of disease (Allwood *et al.,* 2014; Fraga *et al.,* 2011; Hacker *et al.,* 2020).
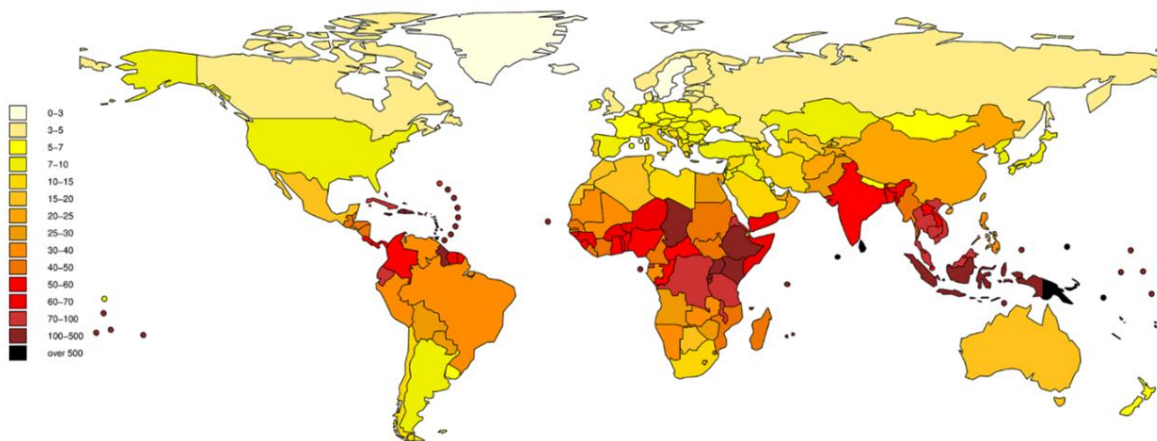
Figure 2:   **Map showing worldwide distributions of leptospirosis (Torgerson *et al.,* 2015)**

The actual leptospirosis burden is not well understood worldwide, and perhaps is among the zoonosis leading to cause serious infections (Chiriboga *et al.,* 2015; Costa *et al.,* 2015). Recently, leptospirosis was named as essential for nearly 2 million people mortality and 500 000 morbidities each year as a result of Disability Adjusted Life Years (DALY) (Torgerson *et al.,* 2015; Xu & Ye, 2018). In the area of multiple circulating febrile illnesses such as malaria,

typhoid, and dengue, leptospirosis has been constantly misdiagnosed and underestimated (Fish-Low *et al.,* 2020; Le-Turnier *et al.,* 2019; Noda *et al.,* 2020). Little awareness among the health practitioners to hardly inadequate laboratory facilities for disease diagnosis are many of the reasons posing leptospirosis remains underreported in developing countries (Pakoa *et al.,* 2018; Rahelinirina *et al.,* 2019; Sandhu *et al.,* 2020).

Despite limited evidence of leptospirosis data both in humans and as well as in animals, seropositivity and prevalence of leptospirosis have been described in various hosts worldwide. In east Africa, a brief policy published in Tanzania highlighted seroprevalence being 30% in humans, cattle (5-51%), pigs (41%), dogs (39%), cats (14%), goats and sheep (38%), wild rodents (17%), bats (19.4%) and 54% in freshwater fish (Mgode *et al.,* 2017). In Uganda, seropositivity of 27.8% was reported in slaughterhouse cattle (Alinaitwe *et al.,* 2020) whereas, 13.4% of workers in a mixed slaughterhouse of sheep, cattle, goats, and pigs were reported in Kenya (Cook *et al.,* 2017).

A distinct number of populations including, livestock, wild animals and humans can harbor more than 200 pathogenic *Leptospira* (MSD, 2006). The leptospiral host can be classified into either accidental or maintenance hosts depending on the infecting serovars. Maintanance host is the one capable of acting as a natural source of infaction for its own species while incidental host is normally not acting a natural source but can be infected (Yadeta *et al.,* 2016) (Table 2).

Table 2: **Maintenance and accidental hosts for veterinary and medical important serovars of *Leptospira* interrogans**

Serovars	Maintenance host	Accidental host
L. Bratislava	Pig	Horse, dog
L. Canicola	Dog	Pig, cattle
L. Grippotyphosa	Rodent	Cattle, pig, horse, dog
L. Hardjo	Cattle	Human
L. Icterohaemorrhagiae	Brown cat	Domestic animals and human
L. Pomona	Pigs and cattle	Sheep, horse, dog

2.4   *Leptospira* and Leptospirosis in Tanzania

Tanzania like many other countries in the tropics and subtropics favors a wide range of fauna of either maintenance or accidental hosts for leptospires (Dreyfus *et al.,* 2016; Hacker *et al.,* 2020). However, a number of studies have been published on the subject leptospirosis in Tanzania is more apparent and given a little priority to absolutely neglected (Biggs *et al.,* 2013). Previous studies have shown leptospirosis is persistent in both humans, cattle, wild and aquatic animals (Assenga *et al.,* 2015; Maze *et al.,* 2018; Mgode *et al.,* 2014). A first cross-sectional study in the 1990s revealed leptospiral seropositivity in humans (0.3%), cattle (5.6%), dogs (38%), and rodents (1.9%) in Tanzania's selected area (Machang'u *et al.,* 1997). Subsequently in the year 2007-2008, a population study based on hospitalized patients in the northern part of Tanzania marked 8.8% seropositive. Similarly, it has been estimated a high annual incidence of 75-102 per 100 000 people annually (Biggs *et al.,* 2013). With the same study approach, an additional study in Moshi, Kilimanjaro was estimated 11-18 annual incidences per 100 000 people per year (Maze *et al.,* 2016).

Given a scarcity of surveillance and appropriate control strategies against leptospirosis, the disease has continued to spill over to aquatic life (Mgode *et al.,* 2014). An epidemiological survey to understand the dynamic transmission of leptospirosis in different habitats is the key to appropriate preparedness of disease prevention and control approaches (Hamm *et al.,* 2015; Shivakumar *et al.,* 2008). Leptospirosis epidemiology in Tanzania is little known however, the disease has been demonstrated in numerous reservoirs including livestock and wild animals (Muller *et al.,* 2016; Schoonman & Swai, 2009). Domestic ruminants predominantly cattle, are often found with leptospires since are vital maintenance hosts for several serovars such as Hardjo, Pomona, and Grippotyphosa (Scolamacchia *et al.,* 2010; Villanueva *et al.,* 2016). This poses risk to humans as an occupational hazard especially to farmers, abattoir workers, and meat vendors (Mirambo *et al.,* 2018).

2.5   Transmission and Clinical Presentation of Leptospirosis

Transmission of leptospirosis is complex with many animal host as well as human interactions (Fig. 3). Many hosts are involved in disease transmission including livestock, wildlife, and humans (Mgode *et al.,* 2014). Rats are often vital reservoirs and determinants for leptospirosis to many maintenances and accidental hosts (Sumanta *et al.,* 2015). In livestock farming, many

species are affected by leptospiral pathogens globally including cattle, dogs, pigs, goats, sheep and can act as a source of infection in humans (Salgado *et al.,* 2014).

Leptospirosis can either spread directly or indirectly (Musso & La-Scola, 2013). Directly is through urine contact from infected animals (Altheimer *et al.,* 2020). Indirect transmission of leptospirosis in livestock is through water, mud, or consuming contaminated animal feeds with urine from carrier animals during grazing particularly on large farms (Jittimanee & Wongbutdee, 2019). Venereal transmission has also been observed in animals (Ryan *et al.,* 2012). The disease is actively associated with reproductive wastage through high abortions, stillbirths, retained placenta, infertility, and decreased milk production in cattle (Baker, 2004; Salgado *et al.,* 2014). Though asymptomatic cases can be seen in cattle and pigs for the infecting *Leptospira* spp. serovars Hardjo and *Leptospira* spp. serovars Pomona respectively (Cook *et al.,* 2017). Leptospires can persist in the urinary and genital tracts for a long period with no clinical symptoms while shedding via urine (Calderón *et al.,* 2014). Maintaining the pathogen in the urinary and genital tract with no clinical symptom accelerates the spread of the disease particularly when introducing new animals into the herds without screening and in co-grazing practice in pastoralists (Acestor *et al.,* 2012). Figure 3 showing transmission and pathological process of leptospirosis, symptom, diagnosis and treatment.
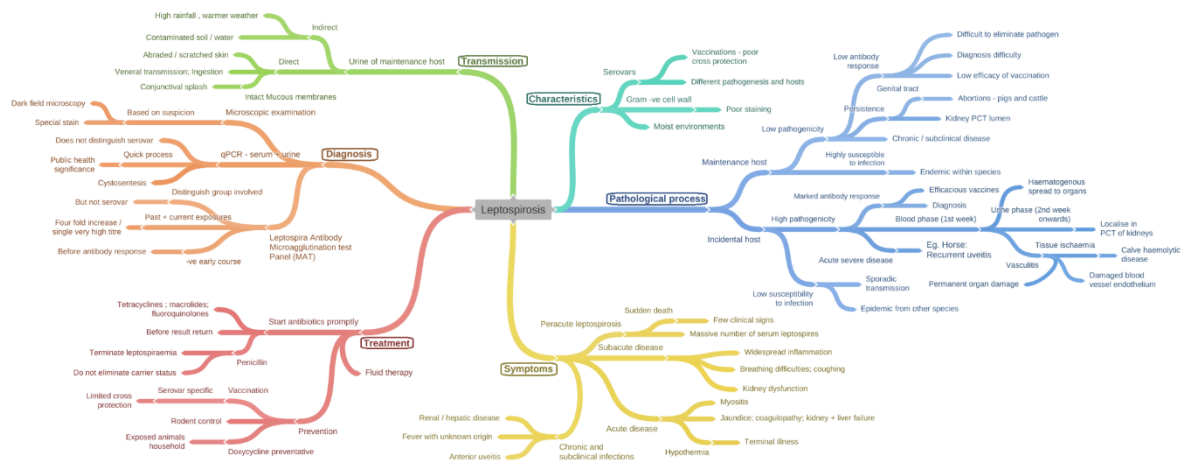
Figure 3: **Illustration showing leptospirosis transmission, pathological process, symptoms, characteristics of *Leptospira*, diagnosis and treatment**

2.6   Disease Prevention and Control

2.6.1   Antimicrobial Therapy

Antimicrobial therapy for clinical animals or people is the first stage of controlling the disease. The commonly used antibiotics are dihydrostreptomycin and oxytetracyclines (Yaakob *et al.,* 2015). However, treatment can be done in parallel with vaccination of the herds to reduce the risk and spread of the disease (Adugna, 2016).

2.6.2   Active Vaccination Control

Vaccination programs are promising and feasible strategies to control leptospirosis both in people and animals. Animal vaccination is important to prevent the shedding of leptospires in urine and reduce the risk of disease outbreaks (Okosun *et al.,* 2016). Though before implementing vaccination is essential to establish the serovars panel that circulates in a specific geographical area for appropriate vaccine use (Goarant, 2016).
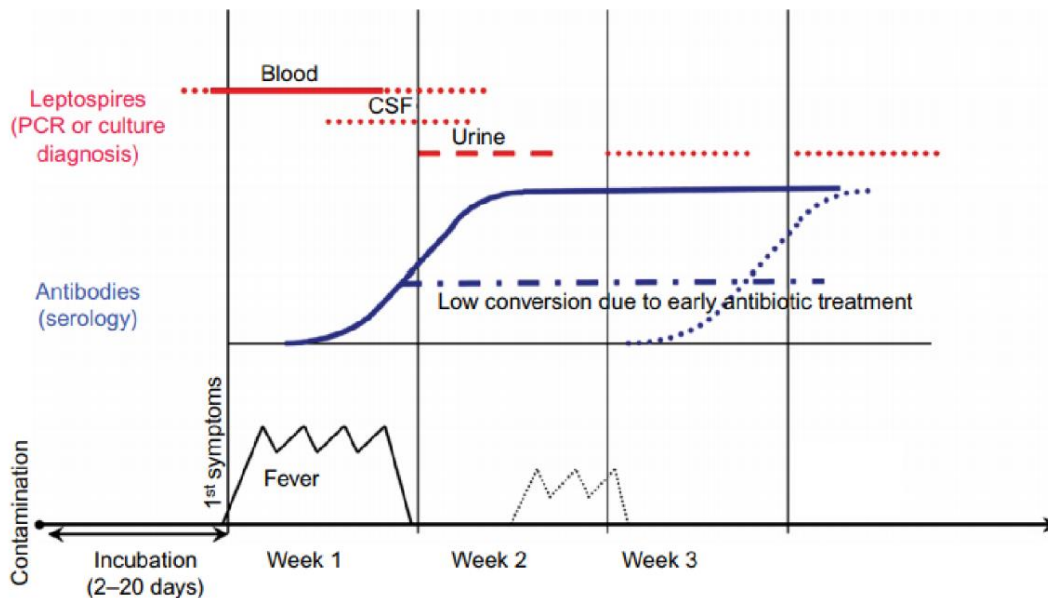
Figure 4:   Fundamental principles underlying the biological diagnosis of leptospirosis

Most developed vaccines require a booster to re-activate their protection at least every year (Spiri *et al.,* 2017; Wang *et al.,* 2007; Xu *et al.,* 2018). However, vaccination alone does not mean bacteria will not be shed into the environment, thus multi-faceted approaches must be considered for controlling the disease (Sonada *et al.,* 2018). A significant achievement has been shown in some countries including New Zealand where the Lepto 3-Way® vaccine was used

to vaccinate cattle (Fig. 5). The vaccine contains serovars Hardjo, Copenhagen, and Pomona that are predominantly infecting the livestock population (Control, 2015). Similarly, Ireland developed also the Spirovac® vaccine for *Leptospira borgpetersenii* serovars Hardjo and "L" Vaccine for *Leptospira interrogans* serovars Hardjo (MSD, 2006).
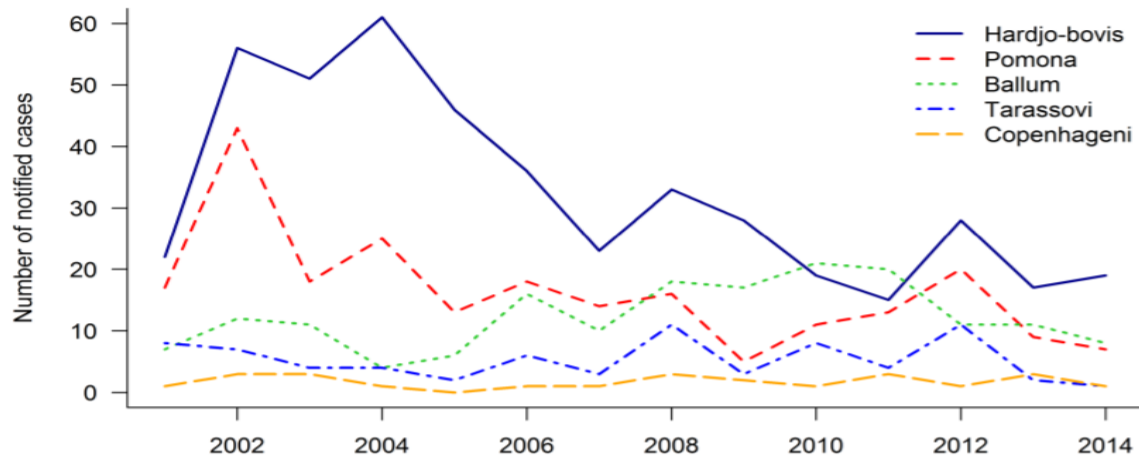
Figure 5: **Showing the success of vaccination program to control leptospirosis in cattle, year 2002-2014 in New Zealand**

2.6.3 Rodent Control

Rodents are the most important natural reservoir for leptospirosis infection in humans and animals (Adugna, 2016; Bharti *et al.,* 2003). Active control of rodents by poisoning or trapping and killing may help to reduce disease spread. This should be continuous practice after identifying areas where rodents live (De-Araújo *et al.,* 2013) may reduce the incidence of leptospirosis infection in humans and livestock (Control, 2015), However, this must be routinely exercised as rodents rapidly recover from inhabitants (Shilova *et al.,* 2009). Rodent elimination by using traps and rodenticide in the environment may be limited in terms of resource allocation from the family level to the government (Minter *et al.,* 2018).

2.6.4 Prevention in Humans

Awareness creation on biosecurity measures is important to protect farmers who are at high risk of infection. Wearing protective equipment such as boots and gloves during handling animals, helping animals from giving birth, burying the placenta, or aborting a fetus (Abdullah *et al.,* 2019; De-Araújo *et al.,* 2013) are avoidable in such circumstances. Bathing or soaking barefoot in mud while having leg or foot wounds/scratches allow easy entry of pathogens into

the body (Desvars *et al.,* 2013). It is important to avoid swimming (Shivakumar *et al.,* 2008), soaking, or bathing in ponds that are contaminated with the urine of infected animals (Benavidez *et al.,* 2019). Culling of an infected animal is found a prerequisite to preventing disease transmission to humans and other animals. Covering food in a clean container to limit rodent access and avoid eating or drinking while carrying wastes (Abdullah *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1   Description of the Study Area

The study was conducted in two agro-ecological zones; the northern and southern highland zone. These are the administrative zones selected based on the fact that are economically important region represent over 70% of improved dairy cattle and milk producers in Tanzania (Njombe *et al.,* 2011). The northern zone (Kilimanjaro, Arusha and Tanga) had more than 3 686 085 indigenous in total and 252 554 improved dairy cattle while the southern zone had more than 8 103 232 indigenous cattle and 35 007 improved dairy cattle population (Njombe *et al.,* 2011). Both the northern and southern highland zones (Mbeya, Iringa and Njombe) form the core milk production of the dairy industry in Tanzania (Njombe *et al.,* 2011), with 6% of the growth rate per annum in the country (Swai & Karimuribo, 2011). A substantial number of the dairy farmers in Tanzania are classified as smallholder dairy farmers of which 90% are from rural and peri-urban areas and are responsible to feed the urban population (Maziku *et al.,* 2017).

The northern zone lies between latitude 3°51′41.40″S and longitude 36°59′44.16″E and occupies four (4) regions namely; Arusha, Kilimanjaro, Manyara, and Tanga. It covers a total area of 125 455 km^2 (Arusha 34 526 km^2; Manyara 50 819 km^2; Kilimanjaro 13 209 km^2 and Tanga 27 342 km^2) corresponding to 13.3% of the Tanzania mainland (Minister, 2013).

The southern highland lies between latitude 9° 10′ 0.12″S and longitude 34° 31′ 0.12″E and occupies six regions namely; Mbeya, Njombe, Iringa, Rukwa, Ruvuma, and Songwe. The total area is 206 921 km^2 (Mbeya 35 954 km^2, Njombe 21 347 km^2, Iringa 35 503 km^2, Songwe 27 656 km^2, Rukwa 22 792 km^2 and Ruvuma 63 669 km^2). However, for this study three regions (Mbeya, Iringa, and Njombe) were considered.
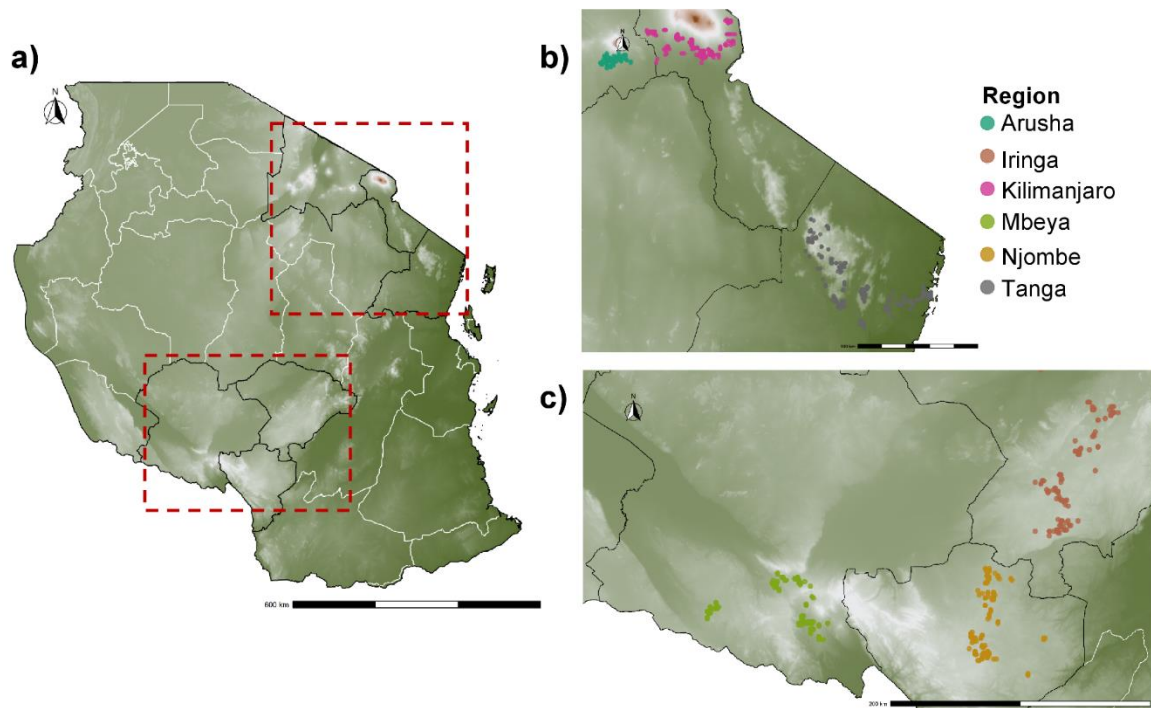
Figure 6:   Geographic location of farms, regions, and dairy zones in Tanzania

Figrure 6 shows a) the geographic location across six regions from two economically important dairy zones over an elevation map of Tanzania. Red squares indicate the important dairy zones, b) a close-up of the northern zone integrated by the regions of Arusha, Kilimanjaro and Tanga in which a total of 12 districts were sampled, c) a close-up of the southern highland zone of Tanzania integrating the Iringa, Njombe, and Mbeya regions in which 11 districts were sampled. In all panels, farm location (dots) is colour-coded to indicate their administrative region.

3.2   Study Design and Target Population

The study was a cross-sectional epidemiological design among identified of improved dairy cattle populations from the six selected region of Tanzania. Samples were collected purposively where the list of cattle population was available from cattle registry of the Africa Dairy Genetics Gains (ADGG) program at the International Livestock Research Institute implemented in Tanzania. Cattle (n=50 000) had previously been enrolled in the ADGG program and smallholder dairy farmers participated in monthly data collection activities related to animal production. Of these 4000 cattle were eligible for sampling and had known genetic characteristics and could be identified by their preliminary information such as an ear tag number, age, and sex from the list of cattle registry.

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3.3   Study Sample Size

In a previous publication in wildlife interface from Katavi region (Muller *et al.,* 2015), the prevalence of the *Leptospira* antibodies in domestic ruminants was estimated to be 30.37%. Therefore, sample size estimation was calculated based on the formula described by Arya *et al.* (2012).

$$n = \frac{(Z^2) P (1-P)}{d^2}$$

Where;   n = number of animals

Z = z score for the level of confidence

P = expected prevalence

d = allowable marginal error

P = 30.37%

Z =1.96 at 95% Confidence interval (CI)

d = 5%

$$n = \frac{(1.96^2) \, 0.3037 \, (1 - 0.3037)}{0.05^2}$$

n = 325

The minimum number of animals recruited for a sample collection from each region was 325 making a total of 1950 minimum sample from all six regions (Arusha, Kilimanjaro, Tanga, Mbeya, Iringa and Njombe). However, more sample were collected best on the availability of the animal list from the cattle registry of the African Dairy Genetic Gains program.

3.4 Selection of Regions, Districts, And Households (HH) in the ADGG Program

Briefly, selection of the geological zone for the study was based on the fact that the regions are economically important for improved dairy farming activities. The northern zone (Arusha, Kilimanjaro and Tanga estimated to have more than 252 555 of improved dairy cattle while the southern part (Mbeya, Iringa and Njombe) comprise over 103 306 dairy cattle. However, the list of animals, district from both the northern and southern zone were already identified and available from the cattle registry of the African Dairy Genetic Gains program. The schematic flow of selection of the animals from six region across the country shown (Fig. 7).
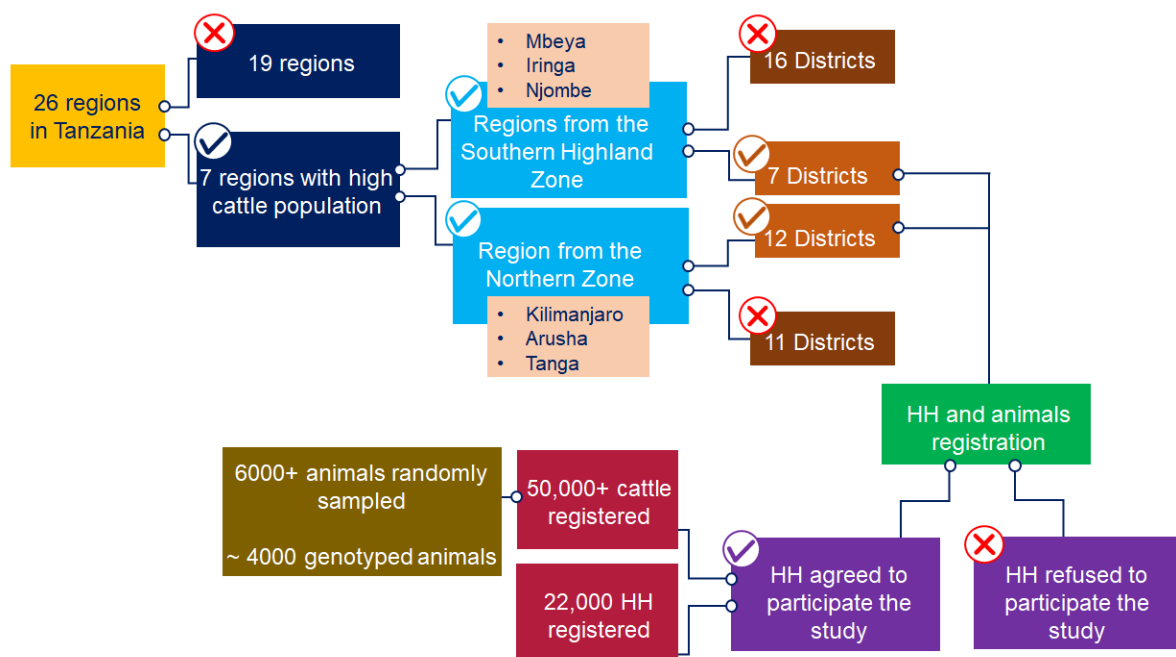
Figure 7: **Schematic flow showing selection procedure of region, district, HH, and animal sampling for animal genotyping in the ADGG program in Tanzania**

Household registration was conducted through participatory rural appraisal (PRAs) by considering the head of household (HHH) willingness to join the program. Registration of HH was done by reconciliation between the HHH and the project team (PRAs) followed by signing consent between HHH and PRAs. For unwilling HHH to join the program were excluded from the study.

3.5 Questionnaire Design and Data

For possible leptospirosis risk factors in smallholder dairy farming, a questionnaire was designed on the Microsoft Office as a template and upload to the Open Data Kit (ODK) cloud

platform software, version 1.22.4 to collect data. It was a semi-structured questionnaire administered to each household/animal/farm care worker. The questionaire in ODK was developed specifying region, districts, wards, farm owner, animal list identifying their ear tag number in assistance by the team and managed at International Livestock Research Institute (ILRI). Before finalizing, a questionnaire was downloaded to android devices which had the ODK app installed via Google Play and piloted in the field (Arumeru district in Arusha) connecting the ADGG cattle registry data set and ILRI ODK cloud server. The ODK form has a graphical user interface (GUI) (i.e Fill bank form, edit saved form, send a finalized form, get blank form and delete saved form) allowing to feed information of one farm at a time and served separately, edit or delete a served form and send all finalized form to the ODK cloud server. On the farm, owners or animal caretakers were interviewed face to face and their answers were recorded onto the form on the app. Among the information collected were demographic variables and herd management data, animal level data, vaccinations, and the presence of rodents, dogs, cats, pigs in or at neighbour farms. Additionally, the geographic coordinates of each farm were recorded. After each day of fieldwork, finalized forms were transmitted securely over the internet and aggregated into one file to the computers of the ODK cloud server at ILRI, Nairobi, Kenya until further cleaning and analysis. Figure 8 shows a systematic flow of data before analysis.
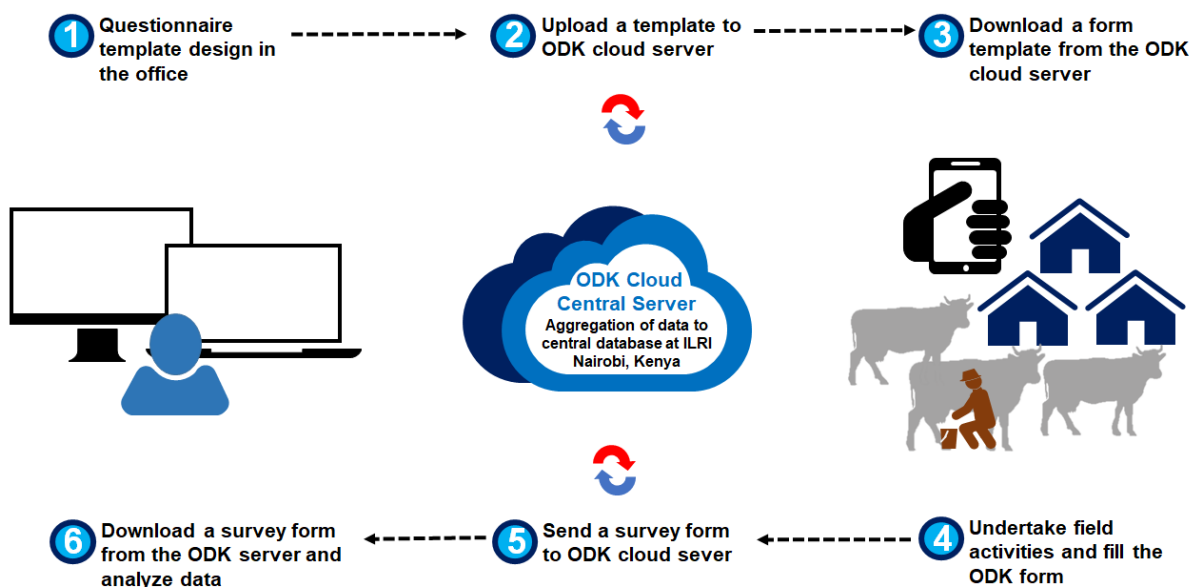
Figure 8:   Design and schematic flow of survey data collection

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3.6   Animal Handling

Cattle were restrained manually to avoid harm or any causes of animal discomfort during sample collection. The exercise was done in compliance with the 2008 Tanzania Animal Welfare Act, part V (Animal Welfare Act, 2008). Using a halter, the animal's head was fastened to an elevated position to allow the jugular vein easily seen. Thoroughly, methylated alcohol was applied to a venipuncture area. Then thumb finger was pressed at the base of the jugular groove to raise blood pressure and visualize the vein by blocking the vein (Geetha & Geetha, 2017). With vacutainer tube and needle's bevel up was firmly inserted into a vein at a 20° angle and blood was drawn into 5 ml EDTA and 10ml plain tube (University Veterinarian & Animal Resources, 2017).

The sample collection was conducted from each household enrolled in the ADGG program and for only animals that underwent genetic testing for their trait characteristics. However, in addition to animals that have unknown genetic traits, they were only sampled if have an abortion history within the last 12 months from the date of sampling.

3.7   Sample Collection

Whole blood was collected into 5ml of Ethylenediaminetetraacetic acid (EDTA) vacutainer tube while blood for serum preparation was collected into 10ml plain (With no anticoagulant) vacutainer tubes. The purpose of collecting whole blood samples was for molecular DNA testing and serum for serological testing antibodies against leptospirosis.

Vaginal swabs were also collected from female animals. The animal's vulva was cleaned with 10% savlon using cotton wool. A swab stick was inserted deep into the vulva and rotated to wet the swabs with vaginal fluid. A wet swab was then emulsified into a 1.5 ml cryotube containing 1ml of phosphate-buffered saline (PBS).

Immediately after sample collection from each animal, all tubes were labeled (date and animal id), barcoded and the sample tube scanned on the ODK form in the tablet device to link the animal biodata. All samples were then kept in a cool box filled with an ice pack (∼ +4°C) before shipping to the laboratory at the Nelson Mandela African Institution of Science and Technology for further storage before analysis.

3.8 Biosafety and Biosecurity Measures in the Field

To ensure biosafety and biosecurity in the field to minimize the spread of infectious disease from farm to farm during visiting farmers, everyone who was involved in animal handling and sample collections was supposed to wear protective gear (coveralls and gloves). After sample collection, all sharps were discarded in a sharp box and other wastes (Gloves, cotton wool) were collected from the area of sample collection and kept in waste bags to leave the place clean. Before moving to another herd/ farm, 10% of virkon sprayed all over the boots to minimize herd to herd spread of diseases.

3.9 Sample Processing and Storage

Upon arrival in the laboratory (The Nelson Mandela African Institution of Science and Technology laboratory for sample collected in Arusha, Kilimanjaro Christian Research Institute for sample from Kilimanjaro, Tanzania Veterinary Laboratory Agency - Vector and Vector-borne Disease in Tanga, Tanzania Veterinary Laboratory Agency - Iringa Center while Mbeya and Njombe a small laboratory set up was planed at district offices provided), all blood samples collected in plain vacutainers were processed on the same day or left at 4°C overnight and processed the next day. Blood was then centrifuged at 3000 revolutions per minute (rpm) for 15 minutes to separate serum from blood cells. The serum was aliquoted using a disposable pasteur pipette into a 1.5 ml ring cap cryovial. All cryovials were respectively labeled to link the parent tube from the field (date and animal id) and then stuck with new laboratory barcodes to link the field barcode with animal biodata and household forms. The barcoded tubes were arranged accordingly in the cryo boxes slot by scanning into a Microsoft Access 2013 database. Finally, samples in the cryo boxes were frozen at -20°C before analysis. Samples collected from Arusha were processed and stored at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory while those from Kilimanjaro, were processed and stored at Kilimanjaro Clinical Research Institute (KCRI). Those from Tanga were processed and stored at Tanzania Veterinary Laboratory Agency-Vector and Vector-borne Disease (VVBD) based in Tanga and from southern highland zone, samples were processed and stored at TVLA Centre at Iringa. Finally, all these samples from Kilimanjaro, Tanga and Iringa were shipped to NM-AIST under a cold chain and stored at -20°C before laboratory analysis. All samples (serum and vaginal swabs) collected were examined by ELISA, MAT and RT-PCR.

Figure 9: Showing sampling strategies and approach of the study to smallholder dairy farmers

3.10   Laboratory Analysis

3.10.1 The ELISA Test for *Leptospira* Hardjo

Serological analysis for leptospirosis was performed at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory in Arusha, Tanzania. The Linnodee *Leptospira* Hardjo ELISA Kit[TM] (Linnodee Animal Care, Oakmount, Holestone Road, Ballyclare, Northern Ireland BT39 0TJ) was used as a qualitative assay to test antibodies against lipopolysaccharide epitopes that are commonly found on the envelope outer surface of *Leptospira borgpetersenii* and *Leptospira interrogans* serovar Hardjo. It was a double sandwich ELISA for L. Hardjo-specific antibodies detection from bovine serum or bulk milk samples. The Hardjo lipopolysaccharide-specific monoclonal antibody bound the Hardjo antigen in a pre-coated plate mobilize test antibody from sera.

The ELISA procedure was performed according to the manufacturer's instructions (Fig. 10). Briefly, 98 µl of diluent buffer was pipetted into a Hardjo antigen pre-coated plate (A1 to H12 wells). Two microlitres (2 µl) of positive, negative controls provided by the manufacturer and blank controls were included in duplicates in well A1, B1, C1, D1, E1 and F1, respectively. The rest of the wells, 2µL of test sera, and controls (i.e positive and negative control) were immobilized Hardjo antibodies into microwells accordingly to make a 2:100 dilution of each test sample. The plates were sealed and incubated for 40 minutes at 37°C with agitation (180

rpm) and washed 4 times with washing buffer to remove unbound antibodies from test sera. Thereafter, 100 µL of peroxidase-conjugated antibody was added into each microwell (A1 to H12), plates sealed and incubated for 30 minutes at 37°C with agitation (180 rpm), and then washed 4 times with washing buffer to remove unbound excess conjugated antibodies. Finally, 100 µL of a chromogenic substrate (TMB) was added to each well, and plates were incubated at room temperature in a dark place for 10 minutes. The 50 µL of stop solution was lastly added to all wells (A1 to H12) and the quantifiable amount of detectably labeled antibody bound to the matrix was measured at 450nm using the Synergy™ HTX Multi-Mode Microplate Reader.
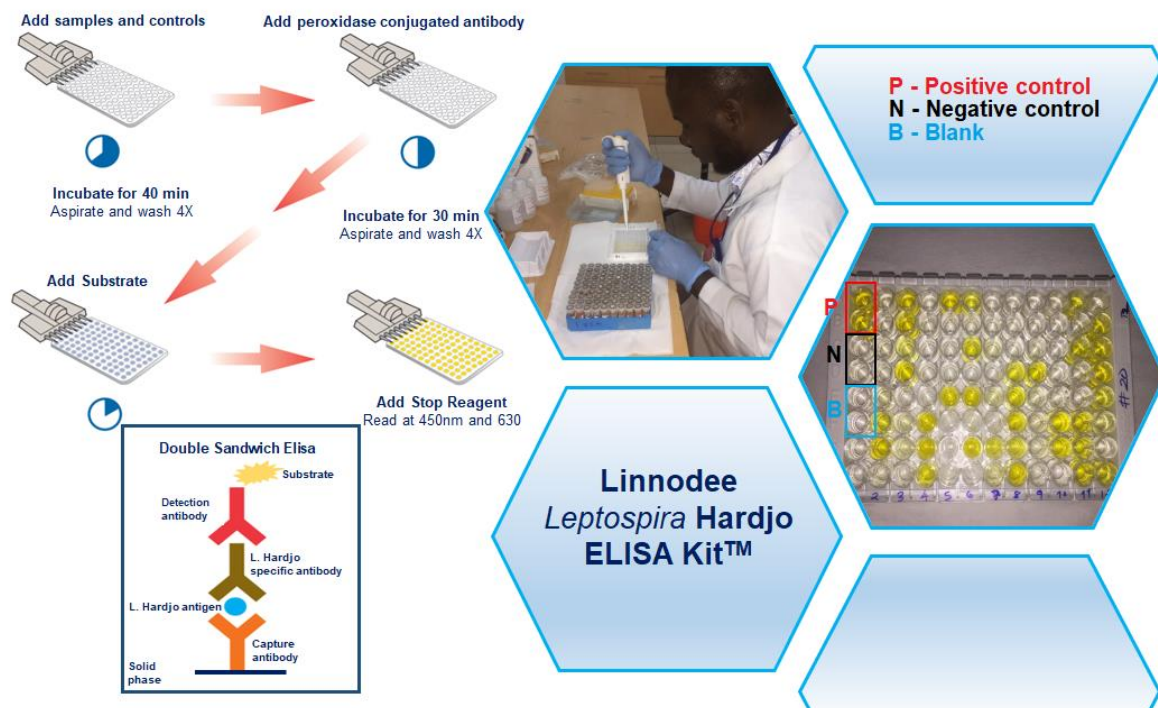
Figure 10:   Laboratory procedure for *Leptospira* Hardjo ELISA test

The optical density (OD) results obtained from the reader were then calculated to positivity ratio (PR) according to the manufacturers. The PR value was obtained by taking the difference between sample OD and mean of negative control OD over the mean of positive control OD and mean of negative control OD.

$$PR = \frac{Mean\ sample\ OD - Mean\ negative\ control\ OD}{Mean\ positive\ control\ OD - Mean\ negative\ control\ OD}$$

Considering positive control, the cut-off point was set at PR > 0.12. At this point, the test assumed 94.1% sensitivity, specificity of 94.8%, and 0.9 kappa index. The results were interpreted in three ways as follows.

PR value	Interpretation
≤ 0.05	Negative
> 0.05 ≤ 0.12	Inconclusive
> 0.12	Positive

3.10.2 Microscopic Agglutination Test

The Microscopic Agglutination Test (MAT) was performed and reference strains plus protocol for serovars serotyping in this study were sourced at Sokoine University of Agriculture (SUA), the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM & BTD) as shown in Table 3. Based on (World Health Organization, 2003), the microscopic agglutination test (MAT) is a reference test for the detection of specific antibodies of any animal species serum against specific live leptospiral antigens. Only five leptospiral antigens were available at ACE IRPM & BTD (Table 3) and were included as leptospiral panel for this study.

Table 3: **List of representative *Leptospira* serovars used in a microscopic agglutination test**

S/N	Serogroup	Serovar	Species
1.	Hebdomadis	Hebdomadis	*Leptospira santarosai*
2.	Icterohaemorrhagie	Sokoine	*Leptospira interrogans*
3.	Australis	Lora	*Leptospira interrogans*
4.	Grippotyphosa	Grippotyphosa	*Leptospira kirschneri*
5.	Pomona	Pomona	*Leptospira interrogans*

All bovine sera were tested for the presence of antibodies against live suspension of *Leptospira* spp. serogroups performed as described previously (Mgode *et al.,* 2014).

Briefly, selected live suspension of *Leptospira spp* serogroups was cultivated in Ellighausen-mcCoullough/Johnson-Harris (EMJH) medium and incubated at 30°C for 5 to 10 days. The

culture was routinely checked for bacterial contamination on the dark-field microscopy till full grown approximately 300×10^8 leptospires/ml of serogroup density that was measured by the MacFarland scale.

Initially, the first row of 96-wells 'U' microtitration plate was filled with 90 µl of pH 7.0 Phosphate Buffered Saline (PBS) and 50 µl to the remaining wells (Fig. 11). Ten (10) µl of a bovine test serum was diluted in 90 µl of pH 7.0 Phosphate Buffered Saline (PBS) to make 1:10 dilution in the first row of 96wells 'U' microtitration plates. Then, 50 µl of 1:10 dilution from the first row of the plate was serially diluted in 50 µl of pH 7.0 PBS. Finally, 50 µl of full-grown selected reference strains *Leptospira* serogroup suspension was then added to all microtiter plate wells doubling the dilution of serum and making 1:20. The mixture was thoroughly mixed on a microshaker and incubated at 30°C for two hours. The antibody-antigen agglutination or antigen clearance was visualized under dark field microscopy and determined the titers.
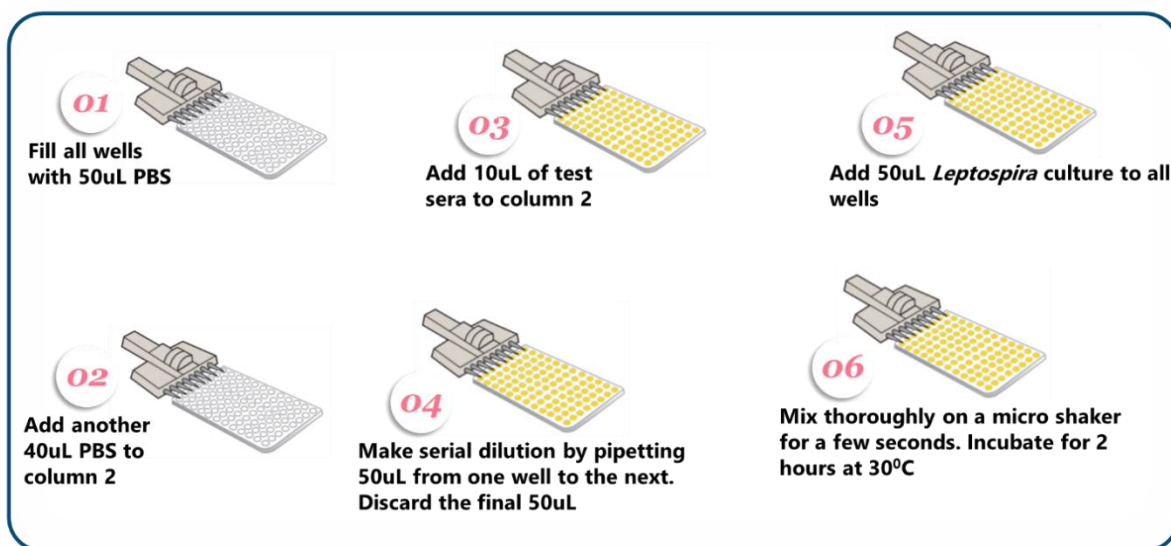
Figure 11:   Steps for microscopic agglutination test

Examination of agglutination was done by transferring one drop of the mixture using a sterilized wire loop to a microscope slide. The endpoint of titer was recorded at that dilution which gives 50% agglutination and left 50% of the cells free. Compare with a control suspension of leptospires diluted 1:2 in PBS without serum in column 1.

A sample was considered positive if 50% or more of the microorganisms in the microtiter well were agglutinated at the titer ≥ 1: 80. This was determined by comparing 50% of leptospires,

which remained free cells with a control culture diluted in phosphate-buffered saline as described by Korver (1992). The positive and negative controls along with samples were titreted at 1:20 for screening and samples that agglutinated more than halfway through were pulled for further titration, as previously described by International Committee on Systematic Bacteriology (Stallman *et al.,* 1984). The samples that agglutinated during screening at 1:20 were further diluted again at dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1,280, 1:2,560, 1:5,120, 1: 10,240 and 1:20,480 to determine the end point titer for each sample. Negative and positive controls were included in each test. Phosphate-buffered saline (PBS) was used as a negative control while rabbit antiserum of each specific serogroup was used as positive control in this study.

3.10.3 The qPCR for pathogenic *Leptospira* spp. Targeting *lip*L32

Molecular analysis of leptospirosis samples was conducted at the International Livestock Research Institute (ILRI), Nairobi, Kenya.

Extraction of genomic DNA (gDNA) was done from vaginal swabs using the DNeasy® Blood & Tissue kit under the manufacturer's directions. A total of 100 µl DNA was extracted from pooled vaginal swabs. Pooling strategies were thoroughly standardized, each pool containing 5 samples of 200 µl in total. From each sample, 40µl was pipetted to make 200 µl of a pool.

A fluorophore-based detection of a TaqMan assay was used to detect the presence of pathogenic *Leptospira* spp. It is a hydrolysis probe designed with specific primers that target a considerable conserved region *lip*L32 gene for pathogenic *Leptospira* spp. (Allan *et al.,* 2018). The aim of using the assay was not to quantify pathogen but it was to identify more circulating pathogenic *Leptospira* other than Hardjo serovars detected from ELISA and MAT.

A two-step qPCR was carried out in a Quantstudio5 system version 1.5.1 software. A total of 20 µL of master mix containing 10 µL of Luna® Universal Probe Master Mix, 0.8 µL of forward and reverse primer with 10µM concentration (*lip*L32-45F5'- AAG CAT TAC CGC TTG TGG TG-3' and *lip*L32-286R 5'-GAA CTC CCA TTT CAG CGA TT-3'), 0.4µL of the probe with 10 µM concentration (*lip*L32-189P 5'-FAM- AA AGC CAG GAC AAG CGC CG-BHQ1-3') and 3 µL of grade water. The DNA amplification set up in the machine was done starting with initial denaturation of 95℃ for 2 minutes followed by 45 cycles of 95℃ for 15 seconds denaturation stage and 60℃ for 30 seconds of annealing and elongation stage. All samples were run in a single well along with one positive template control (PTC), negative control

(NC), and negative extraction control (NEC). Molecular grade water during master mix preparation was treated as negative template control (NTC) while the same molecular grade water used along during DNA extraction was regarded as negative extraction control (NEC) to ensure efficiency and traceability of any cross-contamination in the process from extraction to the final results. The assay was only considered valid if the NTC and NEC did not show an amplification signal.

3.11   Statistical Analysis

All statistical analyses were performed using RGui (64-bit) version 4.0.4 (2021-02-15) and RStudio version 4.1.2 (2021-11-01). To measure associations at animal level, environmental and farm management variables (age, sex, breed, region, water source, herd size, abortion, multiple farm milking practices, hiring bull for breeding, presence of rodent in farm, grazing system, farm to farm distance, education and training by the farmer, farmer's gender, experience in dairy farming, disposal of aborted/placental material, animal body condition score, animal contact with pigs and cat) and the binary ELISA results. Additional environmental data such as population density and solar radiation were sourced from the open.africa, elevation map on USGS, CCI L and Cover LC and the mean annual temperature, precipitation from worldclim.org were included for univeriable analysis. The odds ratios and confidence intervals (0.95 confidence level) estimated using conditional maximum likelihood and normal approximation, respectively, which were implemented in the *epitools* R package (R Core Team, 2021). Further, confidence intervals (C.I) for binomial proportions of seropositive were implemented in *binomCI* function.Variables with statistically significant (p-value $\leq$ 0.05) were further subjected   for multivariate analyses. To model the relationship between our ELISA binomial results and a set of covariates, we built a binomial (logistic) generalised linear mixed effect model (GLMM) with a log link function (Equation 1) implemented in the template model builder *glmmTMB* package (R Core Team, 2021). Continuous fixed effects variables were mean-centered and scaled to standard deviation using the *scale* function. To avoid multicollinearity, Spearman's rank correlation coefficient (rho) tests were run on continuous variables pairs to ensure they were uncorrelated (rho < 0.29 based on Cohen (Jacob, 1992)). A backward stepwise model selection approach was carried out to eliminate one variable at a time based on our model best-fit criteria. For instance, we kept nested models with the lowest Akaike Information Criterion (AIC) and significant (p-value < 0.050) χ^2 statistics from likelihood ratio tests. In parallel, marginal and conditional R^2

calculated using the *rsquaredGLMM* function implemented in the *MuMIn* package was used to select the model explaining most of our data variance. We validated our best model by simulating residuals using the *simulateResiduals* function from the *DHARMa* package. Our model was valid if residuals were plotted versus fitted values and each fixed effect showed no clear clustering patterns

3.12   Ethical Clearance

The Dean, School of Life Science, and Bio-Engineering (LiSBE) at Nelson Mandela African Institution of Science and Technology (NM-AIST) prepared an introduction letter to regional, district level, and local government authorities (LGAs). This was to inform government authorities to know what is ongoing in their administrative areas. Alongside participatory rural appraisal (PRA's), all HH under the ADGG project was reached to introduce the purpose of animal sampling before signing the consent form, interview, and sample collection.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1   Descriptive Results

A total of 2086 out of 4000 animals were sampled from 1370 dairy farms. The reduced number of animals was due to animals being sold, slaughtered or having died. Of these 2086, 15 animals were excluded since they could not be linked to the main ADGG animal registry. The total number of animals sampled per region was Tanga (n = 523), Kilimanjaro (n = 520), Arusha (n = 318), Iringa (n = 305), Mbeya (n = 218), and Njombe (n = 187). The mean age of the sampled cattle was 5.5 years. Of the farms visited, the average animal per herd was 2 and animals were mostly (97.3%) clinically healthy females without udder or reproductive complications. Over 80% of the farms visited were close to the neighbouring farm (within 100 meters) demonstrating intensive farming system with few herds practicing extensive pasture grazing system (distance between farms 100–500 meters). Environmental data set showed a mean annual temperature and precipitation of 19.9°C and 1238 mm, respectively; however slightly variations were present between regions. No farms reported vaccinating against *Leptospira* or any other preventative measures to *Leptospira* infection.

Table 4:   **Distribution of animals and farms sampled in different regions and districts in smallholder dairy cattle in Tanzania 2020**

Zone	Region	District	Number of farms	Number of animals
Northern Zone	Arusha	ACC	82	139
		ARDC	65	79
		MeDC	73	95
	Kilimanjaro	RoDC	38	44
		MoRDC	219	276
		HDC	49	99
		SDC	65	96
	Tanga	TCC	51	132
		MuDC	91	139
		KRDC	60	100
		KUDC	31	44
		LDC	87	106
Southern Zone	Mbeya	MDC	20	25
		MCC	23	27
		MbDC	40	64
	Njombe	MaDC	36	55
		NDC	38	51
		NTC	61	80
		RuDC	80	99
	Iringa	IDC	41	74
		IMC	13	15
		MTC	37	94
		MuDC	57	98

ACC = Arusha City Council, **ARDC** = Arusha Rural District Council, **MeDC** = Meru District Council, **RoDC** = Rombo District Council, **MoRDC** = Moshi Rural District Council, **HDC** = Hai District Council, **SDC** = Siha District Council, **TCC** = Tanga City Council, **MuDC** = Muheza District, **KRDC** = Korogwe Rural District, **KUDC** = Korogwe Urban District, **LDC** = Lushoto District, **MDC** = Mbeya District Council, **MCC** = Mbeya City Council, **MbDC** = Mbozi District Council, **MaDC** = Makambako District Council, **NDC** = Njombe District Council, **NTC** = Njombe Town Coucil, **RuDC** = Rungwe District Council, **IDC** = Iringa District Council, **IMC** = Iringa Municipal Council, **MTC** = Mafinga Town Council, **MuDC** = Mufindi District Council

4.2   Animal Breed Classification

Sampled animals were categorized into four breed types based on their records from the ADGG cattle registry. There were three crossbreed groups including crosses of shorthorn zebu (SHZ) with European breeds such as Friesian (SHZ-X-Friesian), Ayrshire (SHZ-X-Ayrshire) and Jersey (SHZ-X-Jersey), and the fourth group included all indigenous/local breeds. The highest number of animals were SHZ-X-Friesian (n = 1415), followed by SHZ-X-Ayrshire (n = 433), SHZ-X-Jersey (n = 144), and indigenous breed (n = 79).

Table 5:   Distribution of total animal sampled from the ADGG population in smallholder dairy cattle in six regions by breed type, 2020

Breed	Female	Male	Total
Indigenous	72	7	79
SHZ-X-Ayrshire	405	14	419
SHZ-X-Friesian	1358	34	1392
SHZ-X-Jersey	139	2	141
Total	**1974**	**57**	**2031**

4.3   Leptospirosis Awareness Among the Smallholder Dairy Farmers

Of the 1370 total interviewees, 587 respondents were female and 770 male with the majority of respondents having primary education or none 1082/1370 and 275/1370 post-primary school. Although 19.3% and 13.8% of the respondents were aware of tuberculosis and brucellosis as zoonoses that can catch from raw milk consumption, none dairy farmer (0%) was

awere that leptospirosis as a zoonotic disease and it was a new disease to them. However, the majority of respondents (97.44%) consume boiled milk with about one percent (1%) consuming raw milk. Furthermore, 82.7% of the respondents reported burying aborted fetuses and placentas (Table 6). While other farmer throw, leave and feed other animals the aborted material or placenta 6.4%, 1% and 9.6% respectively (Table 6). This justify that there is low biosafety and biosecurity practices among the farmers that may lead into spreading the disease to other animals.

Table 6: **Awareness of respondents on leptospirosis and another milk-borne zoonosis (N=20170), 2020**

A. Zoonosis that can catch from raw milk consumption

Leptospirosis		bTB		Q-Fever		Brucellos is	
n	%	n	%	n	%	n	%
0	0	262	19.1	1	0.07	187	13.7

B. Preparation of milk before consumption

Boiled		Unboiled soure		Boiled soure		Raw		Warm	
n	%	n	%	n	%	n	%	n	%
1334	97.4	12	0.9	1	0.07	10	0.7	12	0.9

C. Management of placenta/arboted materials

Burry		Burn		Throw		Leave		Feed other animals	
n	%	n	%	n	%	n	%	n	%
1133	82.8	2	0.1	88	6.4	14	1	132	9.6

4.4   Serology Results For *Leptospira* spp. Serovars Hardjo

Of the total 2031 tested sera, 13.0% (95% CI 11.6 - 14.5) (n=271) had antibodies against *Leptospira* serovar Hardjo. The seropositivity encountered was significantly related to breed with a higher proportion of indigenous cattle being seropositive, 38.0% (95% CI 27.3 - 49.6) compared to 12.8% (95% CI 11.1 - 14.7) in SHZ-X-Friesian, 11.3% (95% CI 6.6 - 17.8) in SHZ-X-Jersey and 10.3% (95% CI 7.5 - 13.6) in SHZ-X-Ayrshire (Table 7).

Overall, the seroprevalence in male cattle was 29.8% (95% CI 18.4 - 43.4) which was significantly higher than in female cattle at 12.7% (95% CI 11.2 - 14.2). Cattle aged 5 years or above had a seroprevalence of 17.1% (95% CI 14.6 - 19.8) higher compared to those aged below 5 years which had a seroprevalence of 10.2% (95% CI 8.5 - 12.1) suggesting a strong age related exposure risk and increased likelihood of exposure with age (Table 7). The highest seroprevalence was detected in dairy cattle from Iringa 32.0% (95% CI 26.6 - 37.8) and Tanga 19.0% (95% CI 15.7 - 22.6) regions Fig. 12.
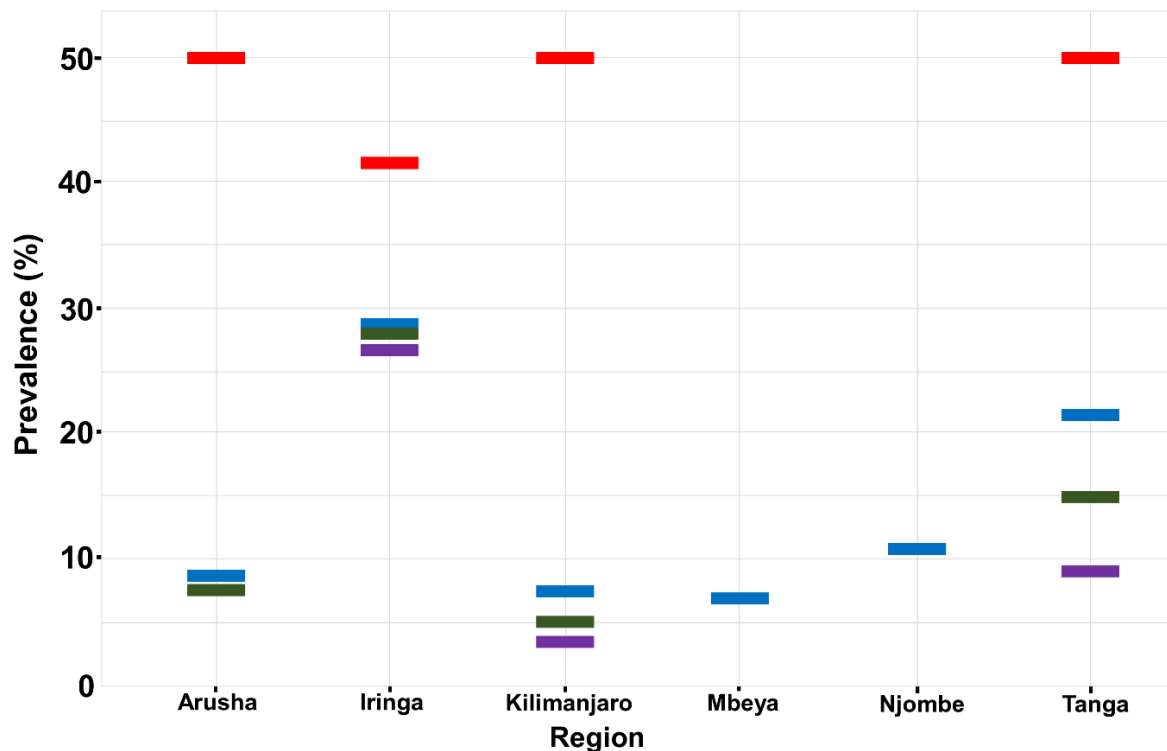
Figure 12: *Leptospira* **serovar Hardjo seropositivity comparisons among the smallholder dairy cattle by animal breed type across the six regions in Tanzania.**

Abbreviation: SHZ-X-Ayrshire (green tile), SHZ-X-Jersey (purple tile), SHZ-X-Friesian (blue tile) and Indigenous (red tile), 2020

The spatial distribution and leptospirosis hotspots in dairy cattle at the district level in the six regions of the northern and southern part of Tanzania are mapped and demonstrated in Fig. 13.
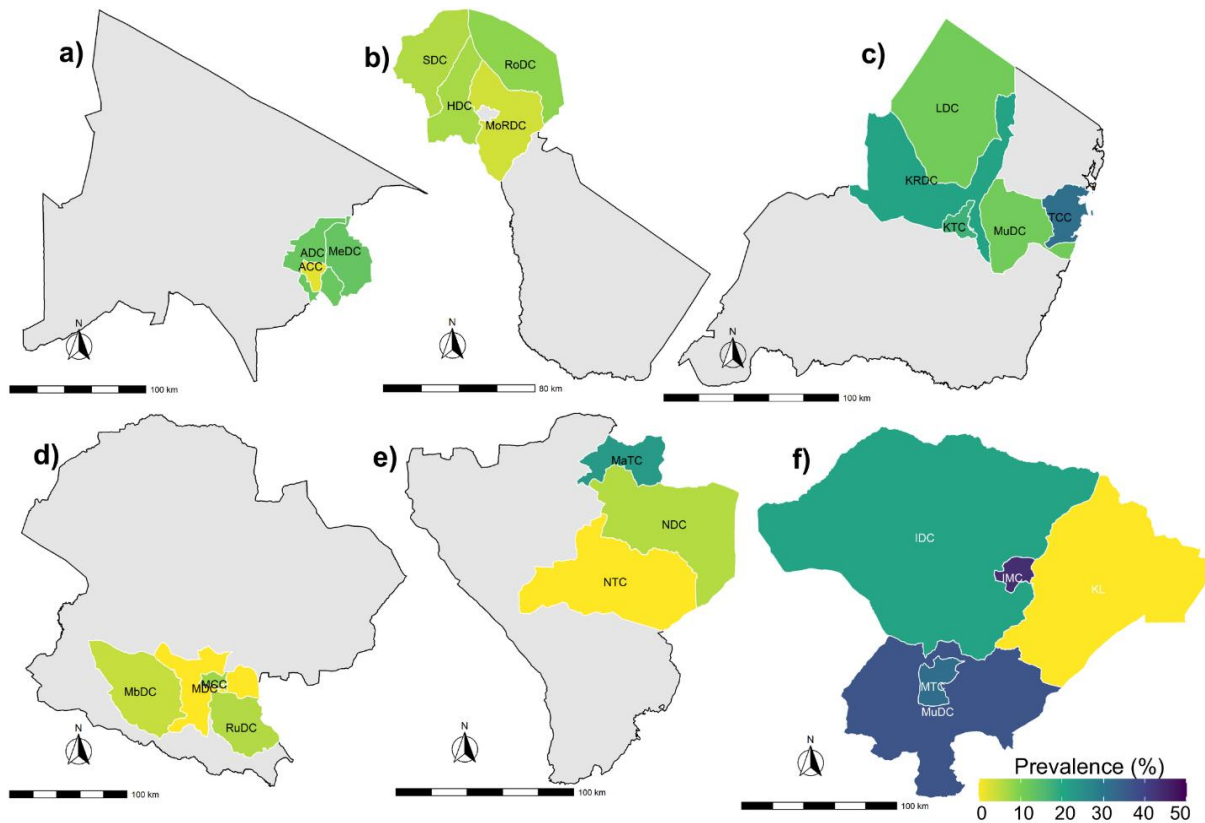
Figure 13: **Geographic mapping of *leptospirosis* distributions and hotspots**

Figure 13 shows the study area of 23 districts across six regions from two economically important dairy zones of Tanzania. The northern zone (A, B and C) and southern zone (D, E and F). a) Arusha region of Arusha City Council (ACC), Meru District Council (MeDC), Arusha District Council (ADC), b) Kilimanjaro region of Rombo District Council (RoDC), Moshi Rural District Council (MoRDC), Hai District Council (HDC), Siha District Council (SDC), c) Tanga region of Tanga City Council (TCC), Muheza District (MuDC), Korogwe Rural District (KRDC), Korogwe Town Council (KTC), Lushoto District (LDC), d) Mbeya region of Mbeya District Council (MDC), Mbeya City Council (MCC), Mbozi District Council (MbDC), e) Njombe region of Njombe District Council (NDC), Makambako Town Council (MaTC), Rungwe District Council (RuDC), Njombe Town Council (NTC) and f) Iringa region of Iringa District Council (IDC), Iringa Municipal Council (IMC), Mafinga Town Council (MTC), Mufindi District Council (MuDC), 2020.

In the region, indigenous cattle showed 50% seropositive in Arusha, Kilimanjaro and Tanga while no seropositive tested in Mbeya and Njombe region. It was only SHZ-X-Friesian tested seropositive against leptospirosis in Mbeya region while the rest breed was negative (Fig. 14).
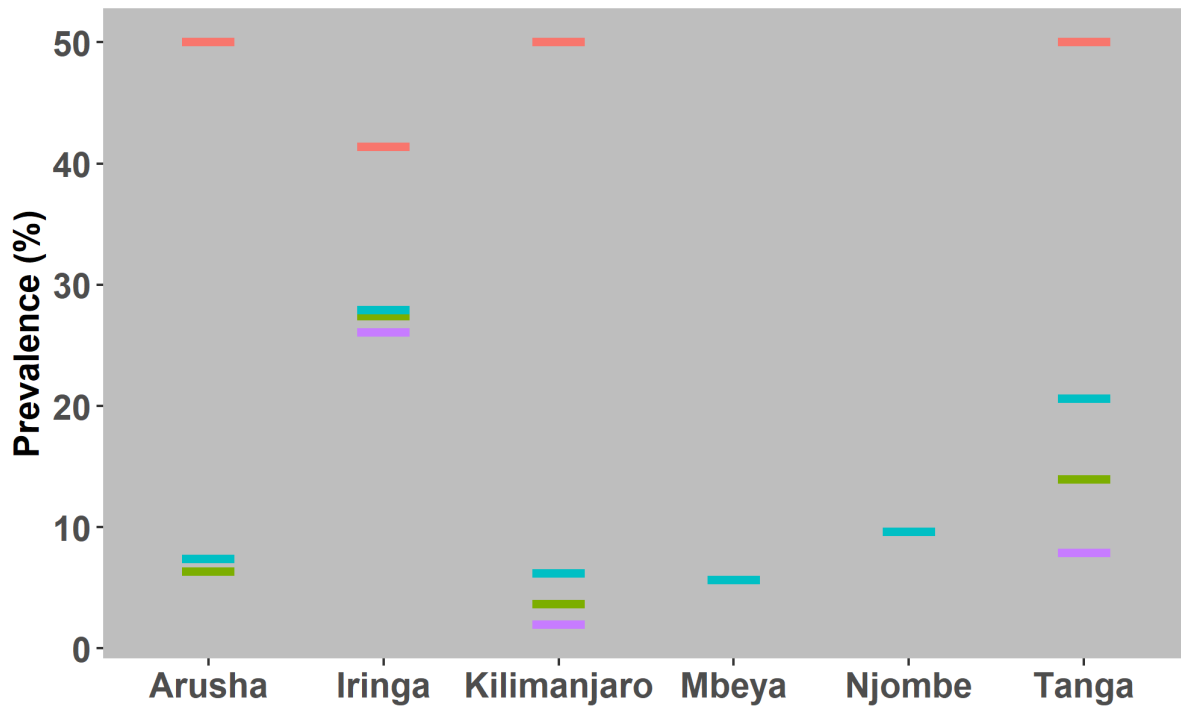
Figure 14: **Comparison of *Leptospira* Hardjo seropositivity in dairy cattle by breed type in each region**

Figure 14 shows the northern (Arusha, Kilimanjaro and Tanga) and southern highland zone (Iringa, Njombe and Mbeya) of Tanzania. Abbreviation: SHZ-X-Ayrshire (green tile), SHZ-X-Jersey (purple tile), SHZ-X-Friesian (blue tile) and Indigenous (red tile), 2020

4.5   Univariate Analyses of ELISA Seropositive Cattle

Twenty-five (25) variables were included at the initial screening to evaluate the relationship with leptospirosis seropositive animals. Eighteen (18) variables were identified as significantly associated with seropositive animals at $p \leq 0.05$. All significant variables were grouped at the animal level, farm management, and environmental variables (regions) and all were further subjected to multivariable analysis Tables 7, 8 and 9.

Table 7: **Univariable associations between *Leptospira* serovar Hardjo seropositive results in dairy cattle and a set of variables at the animal level**

Variables	No. positive animal	Total animal tested	Prevalence (%), 95% CI	OR, 95% CI	p.value
Breed type					
SHZ-X-Ayrshire	43	433	9.93, 7.28 - 13.14	Ref	
SHZ-X-Jersey	16	144	11.11, 6.49 - 17.42	1.13, 0.62 - 2.08	0.75
SHZ-X-Friesian	180	1415	12.72, 11.03 - 14.57	1.32, 0.93 - 1.88	0.13
Indigenous	30	79	37.97, 27.28 - 49.59	5.55, 3.19 - 9.65	0.001
Animal sex					
female	251	2007	12.51, 11.09 - 14.03	Ref	
male	18	64	28.13, 17.6 - 40.76	2.74, 1.56 - 4.80	0.001
Animal age (Years)*					
≤ 5	1041	118	1159	Ref	
> 5	723	149	872	1.8, 1.4 - 2.4	0.001
Abortion in last 12 months					
no	227	1881	12.07, 10.63 - 13.63	Ref	
yes	42	190	22.11, 16.42 - 28.68	2.07, 1.43 - 2.99	0.001

*indicates where variables are not equal to 2071 due to missing data, OR = Odd ratio, CI = Confidence interval, **AI** = Artificial insemination

Table 8: Univariable associations between *Leptospira* serovar Hardjo seropositive results in dairy cattle and a set of variables at the farm management level

Variables	No. positive animal	Total animal tested	Prevalence (%), 95% CI	OR, 95% CI	p.value
Herd size					
≤ 2	73	871	8.38, 6.63 - 10.42	Ref	
> 2	196	1200	16.33, 14.28 - 18.55	2.13, 1.61 - 2.84	0.001
Livestock training					
no	152	1444	10.53, 8.99 - 12.22	Ref	
yes	117	627	18.66, 15.68 - 21.93	1.95, 1.5 - 2.53	0.001
Breeding method					
use AI	136	1508	9.02, 7.62 - 10.58	Ref	
keep/hire bull	133	563	23.62, 20.17 - 27.35	3.12, 2.40 - 4.06	0.001
Animal feeding system					
intensive	171	1767	9.68, 8.34 - 11.15	Ref	
extensive	98	304	32.24, 27.01 - 37.81	4.44, 3.33 - 5.92	0.001
Animal Distance between farms					
≤ 100m	135	1511	8.93, 7.54 - 10.49	Ref	
> 100m	134	560	23.93, 20.45 - 27.68	3.21, 2.47 - 4.17	0.001

Variables	No. positive animal	Total animal tested	Prevalence (%), 95% CI	OR, 95% CI	p.value
Farmer with cats in the farm*					
yes	224	1882	11.9, 10.47 - 13.45	Ref	
no	45	183	24.59, 18.54 - 31.49	2.41, 1.68 - 3.47	0.001
Education					
primary or none	144	1516	9.5, 8.07 - 11.09	Ref	
post primary	125	555	22.52, 19.11 - 26.23	2.77, 2.13 - 3.6	0.001
Gender based farm management					
female	92	835	11.02, 8.97 - 13.34	Ref	
male	177	1236	14.32, 12.41 - 16.4	1.35, 1.03 - 1.77	0.03
Water source					
Tap	143	1319	10.84, 9.21 - 12.65	Ref	
Well	126	752	16.76, 14.15 - 19.62	1.66, 1.28 - 2.14	0.001

*indicates where variables are not equal to 2071 due to missing data, **OR** = Odd ratio, **CI** = Confidence interval, **AI** = Artificial insemination

Table 9: **Univariable associations between *Leptospira* serovar Hardjo seropositive results in dairy cattle and a set of variables at the environmental factor level**

Variable	No. positive animal	Total animal tested	Prevalence (%), 95% CI	OR, 95% CI	p.value
Region					
Mbeya	11	218	5.05, 2.55 - 8.85	Ref	
Kilimanjaro	26	520	5, 3.29 - 7.24	0.99, 0.48 - 2.04	1
Arusha	25	318	7.86, 5.15 - 11.39	1.61, 0.77 - 3.34	0.22
Njombe	16	187	8.56, 4.97 - 13.52	1.76, 0.8 - 3.89	0.17
Tanga	99	523	18.93, 15.66 - 22.55	4.39, 2.31 - 8.37	0.001
Iringa	92	305	30.16, 25.06 - 35.65	8.13, 4.23 - 15.63	0.001

 *indicates where variables are not equal to 2071 due to missing data, **OR** = Odd ratio, **CI** = Confidence interval, **AI** = Artificial insemination

In the final model, ten fixed effects (age, sex, breed, herd size, region, livestock training, farm management, contact with cat and farms distances) and incorporate the dependency among observations by using District, α, as a random effect were included.

$$Yij \sim \text{Bin}(1,\ pij)$$

$$E(Yij) = \sim (pij)$$

logit(pij) = $\alpha + \beta_1$ x Age$_{ij}$ + β_2 x Sex$_{ij}$ + β_3 x Breed$_{ij}$ +

β_4 x Herdsize$_{ij}$ + β_5 x Experience$_{ij}$ + β_6 x Farm_management$_{ij}$ + β_7 x Contact_cats$_{ij}$ + β_8 x Region$_{ij}$ + β_9

β_9 x Livestock_training$_{ij}$ + β_{10} x Distance$_{ij}$ α_i

$\alpha_i \sim N(0,\ \sigma^2_\alpha)$

Where, Yij is the jth ELISA result binomially distributed with a conditional probability, pij, in district i, and $i = 1,\ldots, 20$, and district, α_i, is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2.

A best-fitted model was subsequently simulated by strategies described before (Bates *et al.* 2015). The model was strong for a predictive risk factor in dairy cattle as the KS, Outlier, and Dispersion test gives $p = 0.03$, $p = 0.01$, and $p = 0.49$ deviations respectively (Fig. 15).
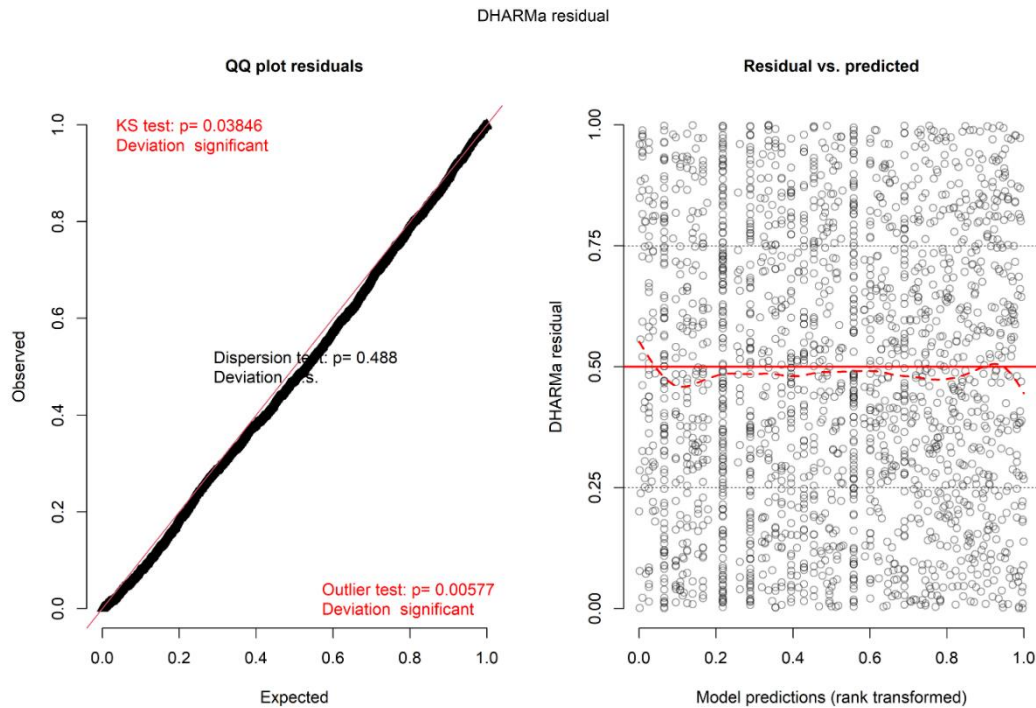
Figure 15: **Simulation plot of the best model for predicting risk factors of leptospirosis occurance in Tanzanian dairy cattle**

The identified risk factors for antibodies to *Leptospira* in cattle from the multivariable model included: age equal to or over 5 years (OR = 1.40, 95%CI 1.03 - 1.90); Indigenous breed (OR = 2.48, 95% CI 1.30 - 4.71) compared to other breeds, farmers with livestock training (OR = 1.63, 95%CI 1.15 - 2.30), herd size greater than 5 animals (OR = 1.67, 95% CI 1.08 - 2.57), male animals (OR= 2.11, 95% CI 1.01 - 4.40), hiring a bull for breeding (OR = 1.69, 95%CI 1.15 - 2.48), farm without cats (OR = 1.68, 95% CI 1.04 - 2.70), animals grazed on pasture (OR = 1.89, 95% CI 1.06 - 3.38) and more than 500 meters distance between the farm (OR = 1.79, 95% CI 1.02 - 3.12). The results of the final multivariable model are indicated in Fig. 18.

4.6 Microscopic Agglutination Results

A total of 2031 cattle sera were tested for the presence of antibodies against five antigen serogroups of leptospirosis. Overall, 13.7% of 2031 tested sera reacted with at least one serogroup of *Leptospira* predominantly serogroup Sokoine 4.8% and Hebdomadis 4.7%. Considering the animal sex, 21.9% of males tested reacted over at least one serogroup while in female animals 12.6% reacted positively to at least one *Leptospira* serogroup. Seroprevalence in different serovars, animal breed type, sex and regions were evaluated at a 95% confidence interval under a normal approximation (Table 10).

Table 10: **Seroprevalence of different 5 *Leptospira* serovars antigen included in the study against breed, animal sex and regions using microscopic agglutination test, 2020**

Variable	Grippotyphosa	Hebdomadis	Lora	Pomona	Sokoine	Total
Breed						
Indigenous	0/79(0)	8/79(10.13)	3/79(3.80)	1/79(1.27)	7/79(8.86)	19/79(24.05)
SHZ-X-Ayrshire	0/433(0)	21/433(4.85)	10/21(2.31)	7/433(1.62)	11/433(2.54)	49/433(11.32)
SHZ-X-Friesian	4/1423(0.28)	61/1423(4.29)	28/1423(1.97)	10/1423(0.70)	76/1423(5.34)	179/1423(12.58)
SHZ-X-Jersey	1/145(0.69)	7/145(4.83)	2/145(1.38)	4/145(2.76)	5/145(3.45)	19/145(13.10)
Region						
Arusha	1/321(0.31)	9/321(2.80)	0/321(0)	4/321(1.25)	10/321(3.12)	24/321(7.48)
Iringa	0/302(0)	30/302(9.93)	15/302(4.97)	14/302(4.64)	19/302(6.29)	78/302(25.83)
Kilimanjaro	2/523(523)	14/523(2.68)	5/523(0.96)	1/523(0.19)	7/523(1.34)	29/523(5.54)
Mbeya	0/217(0)	6/217(2.76)	17/217(7.83)	1/217(0.46)	16/217(7.37)	40/217(18.43)
Njombe	0/189(0)	14/189(7.41)	6/189(3.17)	1/189(0.53)	30/189(15.87)	51/189(26.98)
Tanga	2/526(0.3)	24/526(4.56)	0/526(0)	1/526(0.19)	17/526(3.23)	44/526(8.37)
Animal sex						
Female	4/2014(0.25)	91/2014(4.52)	42/2014(2.09)	20/2014(0.99)	94/2014(4.67)	252/2014(12.51)
Male	0/64(0)	6/64(9.38)	1/64(1.56)	2/64(3.13)	5/64(7.81)	14/64(21.88)
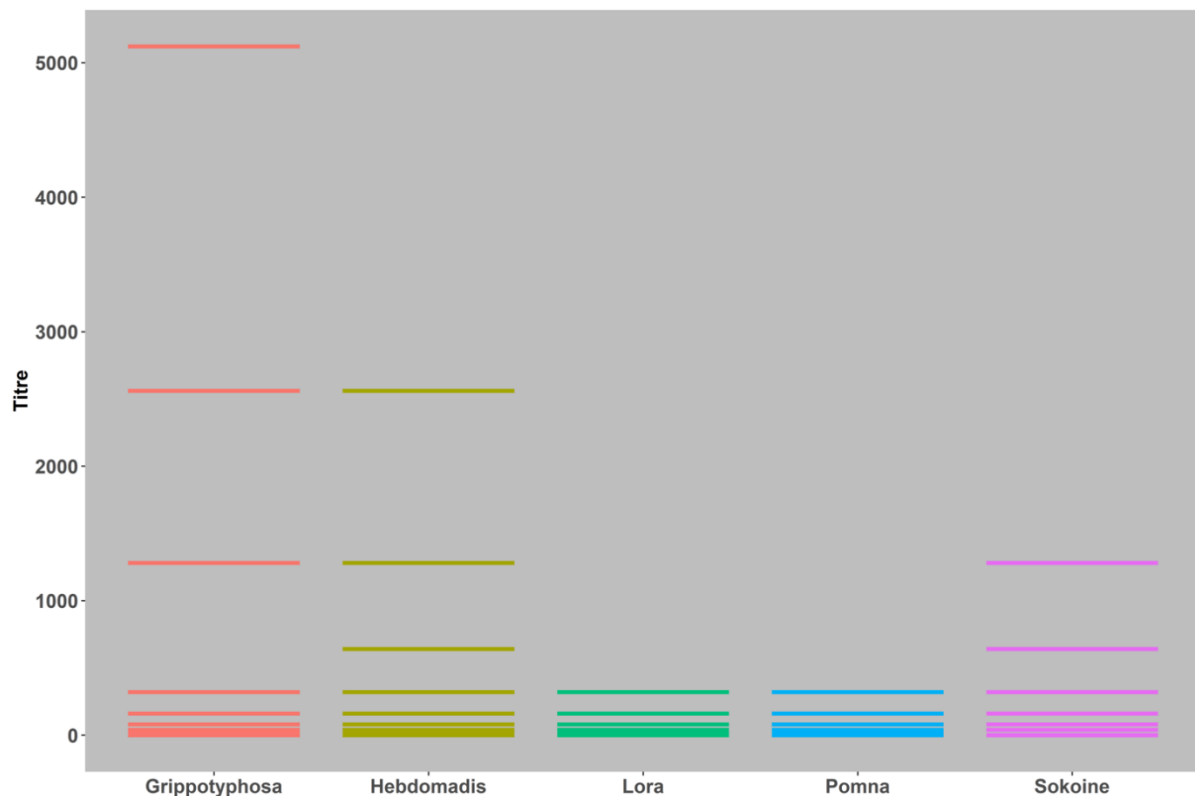
Figure 16: **Heat map of microscopic agglutination test (MAT)**

Figire 16 shows different reacting titre among the serogroup included across seven regions of high small scale dairy farms in Tanzania, 2020.

Twenty out of 2031 animals tested, SHZ-X-Friesian and SHZ-X-Ayrshire showed higher titers (≥1:1000 dilution) at least one *Leptospira* serogroup out of five strains included. In particular, SHZ-X-Friesian had a titer of ≥ 1:1000 dilution against three serogroups (two against Sokoine, twenty-one against Grippotyphosa and two against Hebdomadis), while SHZ-X-Ayrshire tested had a titer f ≥ 1:1000 against two serogroups (three animals against Grippotyphosa and one animal against Hebdomadis). A Detailed heat map of most reacting titer against different animal breed tested against five serogroup (Fig. 16).
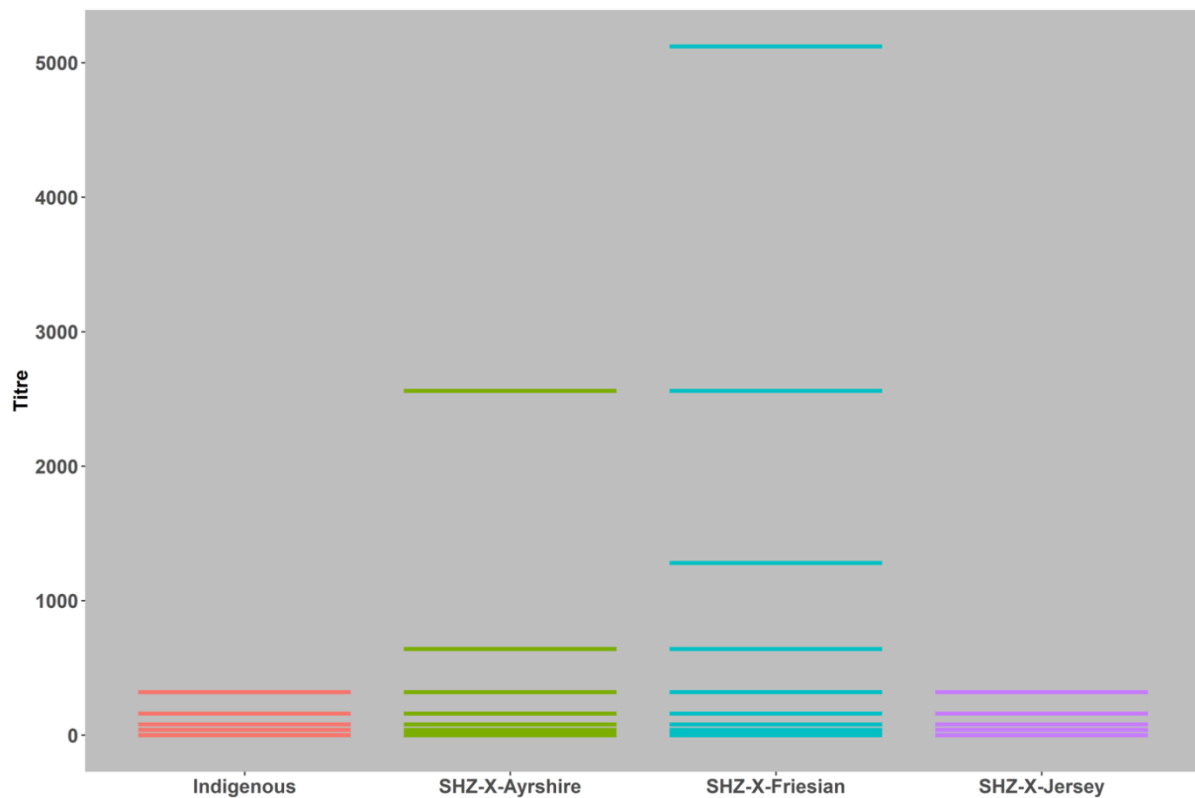
Figure 17:   Heat map of microscopic agglutination test (MAT) to the high reacting titre in different animal breeds, 2020

4.7   The qPCR results for lipL32 pathogenic *Leptospira* spp.

Of 2031 animals sampled for testing, only 1461 swab samples from female animals were subjected to PCR test to identify the infected animal with pathogenic *Leptospira* of *lip*L32 gene. The 13.6% (11.8 – 15.4, 95% CI) were tested positive for *lip*L32 gene pathogenic *Leptospira* spp. Of these 1461 gave positive results were compared the prevalence between breed, region, age and herd size by Fisher's Exact Test, $p < 0.05$. With a breed wise, 22.6% (12.28 - 36.21, 95% CI) were Indigenous, 13.6% (10.2 - 17.62, 95% CI) SHZ-X-Ayrshire, 13.47% (11.35 - 15.81, 95% CI) SHZ-X-Friesian and 9.82% (5.01 - 16.89, 95% CI) SHZ-X-Jersey. Njombe 37.97% (27.28 - 49.59, 95% CI) and Iringa 16.75% (11.89 - 22.61, 95% CI) region shown higher prevalence and it was statistically significant for animals tested positive for *lip*L32 gene with the OR = 5.44 (3.17 - 9.32, 95% CI) and OR = 1.79 (1.12 - 2.85, 95% CI) respectively of $p < 0.05$ (Table 11).

Table 11: **Summary of total sample tested by RT-PCR targeting lipL32 gene for pathogenic *Leptospira* and prevalence by breed**

Variable	Positive	Total	Pr, 95%CI	OR, 95%CI	p-value
Breed type					
SHZ-X-Ayrshire	48	353	13.6, 10.2 - 17.62	Ref	
SHZ-X-Jersey	11	112	9.82, 5.01 - 16.89	0.69, 0.35 - 1.38	0.33
SHZ-X-Friesian	127	943	13.47, 11.35 - 15.81	0.99, 0.69 - 1.41	1
Indigenous	12	53	22.64, 12.28 - 36.21	1.86, 0.91 - 3.79	0.1
Region					
Kilimanjaro	51	504	10.12, 7.63 - 13.09	Ref	
Arusha	37	264	14.02, 10.06 - 18.8	1.45, 0.92 - 2.28	0.12
Iringa	34	203	16.75, 11.89 - 22.61	1.79, 1.12 - 2.85	0.02
Njombe	30	79	37.97, 27.28 - 49.59	5.44, 3.17 - 9.32	0.001
Tanga	46	411	11.19, 8.31 - 14.65	1.12, 0.73 - 1.71	0.67
Age					
≤ 5	123	881	13.96, 11.74 - 16.43	Ref	
> 5	75	580	12.93, 10.31 - 15.94	0.92, 0.67 - 1.25	0.59
Herd size					
≤ 5 animals	157	1179	13.32, 11.43 - 15.39	Ref	
> 5 animals	41	282	14.54, 10.64 - 19.2	1.11, 0.76 - 1.61	0.63

Pr = Prevalence, **OR** = Odds ratio

4.8   Discussion

Over sixty-seven percent of the total samples collected were overrepresented by the SHZ-X-Friesian while three percent of the total samples collected were underrepresented by the Indigenous group. Despite the lower sample represented from the Iringa region, a high percent of seropositive (30.16%) observed perhaps climatic conditions accompanied by other host species such as pigs that carry importance serovars for bovine as well.

Different results obtained between the three diagnostics test used can be attributed to several reasons and preferred diagnostic tests. For RT-PCR, vaginal swabs or urine samples are suitable only if samples are collected after three weeks following infections. At this stage, bacteria have been well established in the reproductive and urinary systems respectively (Balamurugan *et al.*, 2018). Blood can be recommended sample of test before bacterial clearance in blood circulation. Collection of blood is ideal at the early stage of infection before bacterial clearance in blood circulation and it is obvious after three weeks of infection (Goarant, 2016). For MAT and ELISA serum is recommended test at all stages of infections. However, the MAT test can be difficult to differentiate between current and previous infections while ELISA depends on the kit targets IgM or IgG for current or later infections respectively (Niloofa *et al.*, 2015).

In terms of seroprevalence, there was a difference between the regions. Iringa and Tanga for example had higher seroprevalence than the other regions, with 30.16% and 18.93%, respectively. This is backed by prior data in Katavi, which showed a 30.37% seropositive (Assenga *et al.*, 2015) and 30.3% in Tanga (Schoonman & Swai, 2010). The study observed higher seropositive cattle tested from the southern zone 19.8% than in the northern zone 11.4% where parts of the southern zone are characterized by a relatively warm and temperate environment with a short dry season. This may be caused by slightly different climatic conditions such as humidity, precipitation and temperature which are essential factors for viable leptospiral maintenance and dissemination (Budihal & Perwez, 2014; Chadsuthi *et al.*, 2012; Lindahl *et al.*, 2011).

Extensively farming practice were significantly more likely to be seropositive than intensively farming OR = 2.31 (95% CI 1.36 - 3.91). Similarly, cattle on distant farms to farms of more than 100 meters were at higher risk OR = 1.75 (95% CI 1.16 - 2.64) being seropositive than farms of below 100 meters distance. It was observed during the study that farms with increased distance between farms had greater access to pasture. In addition to the farm management practices, low biosafety and biosecurity potentially put dairy cattle at higher risk of leptospirosis and spread in the herd. These findings complement past studies which concluded that pasturing practices and co-grazing encourage pathogen transmission to susceptible animals (Salgado *et al.*, 2014) through contact with infected animals and access to contaminated pastures and water (Schoonman & Swai, 2010).

Meanwhile, varying sources have reported different susceptibility of cattle to leptospirosis infection based on the age class. Older animals are more likely to be seropositive than younger animals due to prolonged exposure to pathogens (Yatbantoong & Chaiyarat, 2019). The study found dairy cattle with the age of equal to or above five (5) years were more likely to be seropositive OR = 1.41 (95% CI 1.05 - 1.90) than younger animals below 5 years as previously described (Olivera *et al.,* 2018). However, of all 1370 study farms included, none of the farms had a history of vaccination, treatment or any control measures against leptospirosis. This suggests that high seropositivity to *Leptospira* serovar Hardjo in older cattle may be due to increased possibility of exposure to *Leptospira* in the environment and carrier animals in the same herd (Ryan *et al.,* 2012).

Livestock training was an important factor for seropositivity in dairy cattle in the study sites. It was interesting that no farmers who had received livestock training, their animals were at higher risk of being seropositive OR = 1.62 (95% CI 1.15 - 2.27) than farmers who did not have or formal knowledge of livestock keeping. The study had expected that farmers with training and or knowledge of dairy management, and their animals could be at lower risk to contract leptospirosis as they abide by recommended farm management practices and precautionary measures to prevent disease spread. This situation could be attributed to numerous reasons but the major reason was that, the most trained farmers hired untrained personnel to take care of the animals instead.
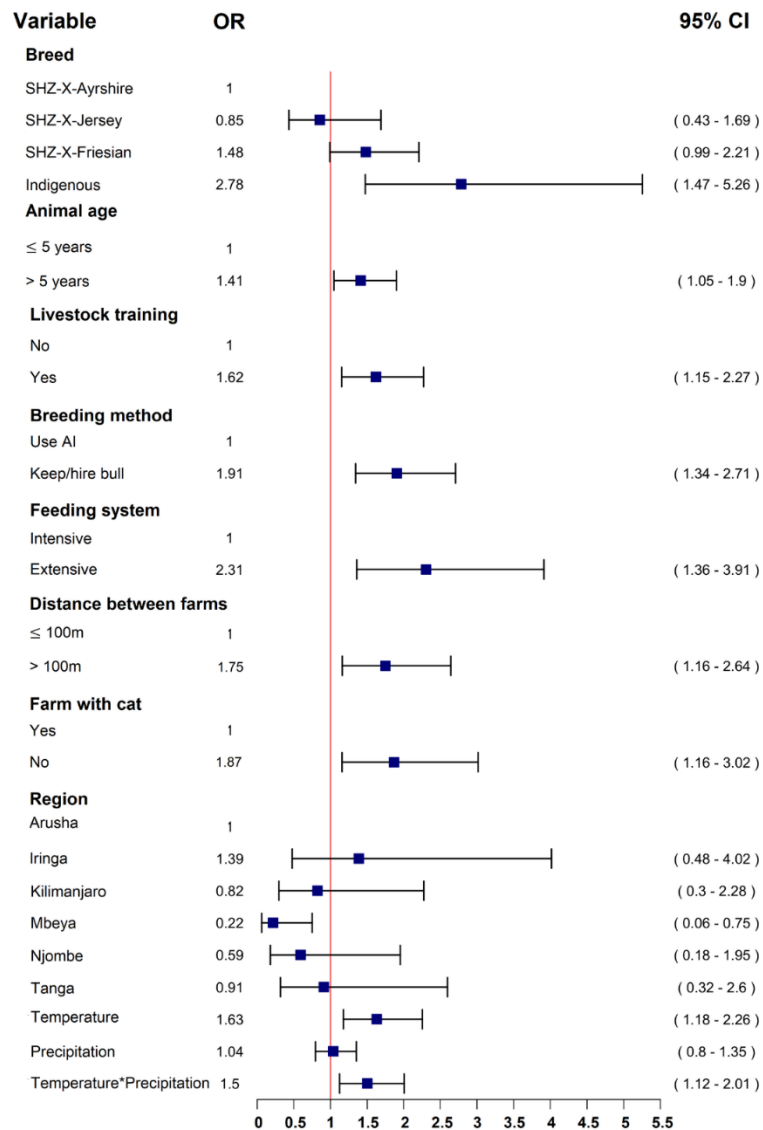
Figure 18: **A glmmTMB forest plot summarizing significant predictive variables for leptospirosis and association to seropositivity in smallholder Tanzanian dairy cattle**

Livestock production in Tanzania remains a challenge particularly for smallholder dairy farmers. In this study, several smallholder dairy farmers relied on breeding with a bull, and a few of them used artificial insemination (AI) which was not easily accessible because of the limited expertise for this service. Cattle on farms with kept or hired bull for breeding were more likely to be seropositive than cattle on farms using AI methods for breeding purposes (OR = 1.91; 95% CI 1.34 – 2.71). Hiring a bull for breeding in Tanzanian dairy cattle was shown to be an important factor for disease spread in animals within the herd, and between neigh bour farms through sexual contact (Boey *et al.,* 2019). It has been reported by previous authors

(Yatbantoong & Chaiyarat, 2019), that hir ing a bull or close contact between animals for calf raising is the most remarkable determinant for leptospirosis infection in smallholder dairy farms.

Moreover, this study found that animal breed was also significantly associated with seropositivity; with indigenous cattle being significantly more likely to be leptospirosis seropositive OR = 2.78 (95% CI 1.47 – 5.26) than Friesian and Jersey crosses [(OR = 1.48 (95% CI 0.99 - 2.21), and OR = 0.85 (95% CI 0.43 - 1.69) respectively].

This study also found that farms that do not keep cats on the farm were significantly more likely to have seropositive cattle OR = 1.87 (95% CI 1.16 - 3.03). Epidemiologically, rodents are a principal reservoir and are known to contaminate pasture, and the environment and consequently, livestock may acquire leptospirosis infection during grazing (Ribeiro *et al.,* 2017). Keeping cats in the was likely to put down the rodent numbers particularly in cow sheds, in the reserved pastures or hay barns; thus it could be a protective measure to reduce incidences of cattle contracting leptospires.

Once again, the findings in this study underpin the importance of leptospirosis in dairy farms. The presence of *Leptospira* spp. in dairy farms was attributed to environmental contamination with the sources of the pathogen and dairy animals that share grazing pastures and the environment (Nthiwa *et al.,* 2019). These may also become sources of infection to animal caretakers or slaughterhouse workers (Zhao *et al.,* 2016). It has already been highlighted that the leptospirosis infection in humans is largely dictated by its prevalence in livestock (Ngugi *et al.,* 2019). Infected animals can contaminate the environment with leptospires by excretion in urine which can remain infectious in the environment for a few weeks to a month (Allan, 2016). Contamination of the environment is linked to spread via water sources or animal feeds that can be accessed by other animal species, particularly in the pastoral area (Pongsumpun, 2011) which causes indirect transmission.

Given that there was no vaccination history against leptospirosis in Tanzanian dairy cattle coupled with the inability of MAT titer to distinguish between current and previous infection (Levett, 2001), it was difficult to conclude seropositive animals from MAT were the current or past acquired infections. Therefore, it is important to distinguish between acute infections from chronic one by including multiple analytical methods from a single sample. This is important for the prioritization of disease and resource allocation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings of this study rectify the widespread leptospiral infection among smallholder dairy cattle across the country based on antibodies against *Leptospira* serogroups detected by ELISA and MAT test in sera of dairy cattle. In all six regions, the number of animals infected and exposed to *Leptospira* is higher.

Serological reactivity (ELISA and MAT) and RT-PCR evidence suggest that the study area is contaminated with pathogenic *Leptospira* spp. the major circulating serogroups were Pomona (serovars Pomona) 15.7%; Icterohaemorrhagiae (serovars Sokoine) 8.98%; Australis (serovar Hardjo) 4.87%; Grippotyphosa (serovar Grippotyphosa) 3.37% and Hebdomadis (serovar Hebdomadis) 1.49%. The result highlights leptospiral infections to Tanzanian smallholder dairy cattle are caused by different multiple serogroups with possible transmission by indirect contact with contaminated water and animal feeds from urine of an infected host. Urbanization and climatic change resulted in the drought that drive livestock movement for grazing search and human-animal interaction eventually leptospirosis spread.

5.2 Recommendations

With evidence of both serological and molecular tests of leptospirosis infection among smallholder dairy cattle in Tanzania, a study recommends additional work on molecular epidemiology of leptospirosis should be carried out in Tanzania smallholder dairy cattle. Also assessment by using both clinical and non-clinical settings of leptospirosis in people, particularly those who are working on dairy farms. A comprehensive epidemiological study on dairy cattle and people will allow a precise estimation of the actual prevalence and the role of dairy cattle on human leptospirosis in Tanzania. Bacterial isolation, molecular typing can be taken under consideration to determine important pathogenic leptospiral strains in the future study to counterpart ELISA, MAT and RT-PCR weaknesses. Understanding important pathogenic leptospiral in humans and livestock will help the Ministry of Health and Social Welfare to consider including a rapid serological test for febrile illness patients in health facilities particularly communities living in rural parts who are at great risk to contract leptospirosis from livestock.

Many studies have reported leptospirosis endemic in Tanzania, the cut-off point value for MAT should be revised to bring to an actual estimation of the disease. Since leptospirosis is a zoonosis therefore one health approach for effective intervention should be used in Tanzania. Also due to climatic change and the emergence of new leptospiral strains, active disease surveillance should be conducted to protect livestock lives, and generate baseline information and disease hotspot of inter-epidemic serovars transmission and risk. Awareness among the physicians and laboratory staff should raise on leptospirosis for better management and prevention of the disease.

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Appendix 1:   **Questionnaire Survey for Measuring Leptospirosis Awareness and Risk Factors in Smallholder Dairy Cattle in Tanzania**

SECTION 1: REGISTRATION	Hint	Label
Region ⊙ Select/filter		region
District ⊙ Select/filter		district
Ward ⊙ Select/filter		ward
Village ⊙ Select/filter		village
Farmer Name ⊙ Select name from list		
Signed consent to allow sampling ⊙ Yes ⊙ No		consenting

If **"NO"** above terminate interview

Interviewer ⊙ Shabani		interviewer
Date Dd/mm/yyyy	Today's date	collect_date

SECTION 2: DEMOGRAPHICS INFORMATION

Interviewee Name

Free text   Free text   interviewee

Gender

⊙ Male   Mark   appropriate
                                  response

⊙ Female

Role in cattle management   cattle_role

⊙ Principle person looking   Mark   appropriate
after cattle owner   response

⊙ Occasionally look after
cattle

⊙ Do not look after the cattle

Level of education   education

⊙ None   Mark highest

⊙ Primary

⊙ Secondary

⊙ Tertiary

How many year's experience keeping cattle?

Number/integer (Enter years)

Have you ever been on a livestock training course for dairy cattle?   livstcktraining

⊙ Yes	Mark appropriate
⊙ No	response

If YES above

		year
What year did you have your training	Enter integer (4 digits) of training year	

Are you aware of any diseases you could catch from your cow milk?		
☒ Bovine TB	Check all listed	milk_zoonoses
☒ Brucellosis	If **"other"** specify	
☒ Q fever		
☒ RVF		
☒ Don't know		
☒ none		
☒		
other…………………(specify)		

Are you aware of any diseases you could catch from an aborted calf?		Abortion_zoonoses
☒ Brucellosis	Check all listed	
☒ Q fever	If **"other"** specify	
☒ Leptospirosis		
☒ Rift valley fever		
☒ Don't know		
☒ none		
☒ other		
☒		
other…………………(specify)		

Which of the following statements best describes this herd role for the owner.		reason_own_cattle
⊙ A primary income source to owner	Mark appropriate response	
⊙ Secondary income source to the owner		
⊙ Just for home consumption and sale to neighbours		
⊙ Only home consumption		

SECTION 3: HERD MANAGEMENT

How many heifers and cows do you currently have in the herd?	herd_size
Enter integer	

Do you keep your own bull for breeding?	
⊙ Yes	bull
⊙ No	

If YES above

Do you hire out the bull to neighbours?	bull_hire
⊙ Yes	
⊙ No	

In the last 12 months have you brought new animals into this herd?	
⊙ Yes	new_animals
⊙ No	

If YES above

Select appropriate

⦿ Market

⦿ Neighbour

⦿ none

Did you do any pretesting?   pretest

⦿ Yes

⦿ No

Do you keep sheep at the same household as these cattle?

⦿ Yes   sheep

⦿ No

Do you keep goats at the same household as these cattle?

⦿ Yes   goats

⦿ No

Do you keep pigs at the same household as these cattle?

⦿ Yes   pigs

⦿ No

Do you keep dogs at the household?

⦿ Yes   dogs

⦿ No

Which option best describes the feeding management

⊙ Only zero grazed	Mark appropriate management response
⊙ Generally zero grazed but occasionally graze at pasture	
⊙ Generally grazed at pasture	

Which option best describes water provision for the herd
		water
⊙ Well/bore hole	Mark appropriate response	
⊙ Tap water		
⊙ River or stream		

Do you vaccinate the herd routinely against any diseases
⊙ Yes	Mark appropriate vaccinations response
⊙ No	

If YES above

FMDV
⊙ Yes	FMDV
⊙ No	
⊙ Don't know	

Brucellosis
	brucella
⊙ Yes	
⊙ No	
⊙ Don't know	

Leptospirosis
	lepto
⊙ Yes	
⊙ No	
⊙ Don't know	

Pasteurella   pasteurella

     ⊙ Yes

     ⊙ No

     ⊙ Don't know

Black leg   blackleg

     ⊙ Yes

     ⊙ No

     ⊙ Don't know

Anthrax   anthrax

     ⊙ Yes

     ⊙ No

     ⊙ Don't know

Other   other_vacc

     Free text

Which option best describes who   milker
milks the cows?

     ⊙ Respondent   Mark appropriate

     ⊙ Owner (if not respondent) response

     ⊙ Family member

     ⊙ Outside milker/contract
     milker

If outside milker

Does the milker go to multiple   milker_farms
farms?

⊙ Yes	Mark	appropriate
⊙ No	response	

Which best describes preparation of milk from this herd before drinking? milk_prep

⊙ Warm up on fire or stove	Mark	appropriate
⊙ Bring to the boil on fire or stove	response	
⊙ Consume without any heating		

Who normally assists with calving for the herd? calving_assist

⊙ Respondent	Mark	appropriate
⊙ Owner (if not Respondent)	response	
⊙ Family member		
⊙ Outside help		

How do you normally dispose of the after birth/placenta after a calving placenta

⊙ Leave for cow to eat	Mark	appropriate
⊙ Burn	response	
⊙ Bury		
⊙ Throw on rubbish heap		
⊙ Feed to other animals (dogs/pigs)		

Has any cow aborted in the last 12 months as far as you are aware? abortion

⊙ Yes

⊙ No

⊙ Don't know

In your view do you have trouble getting cows in calf?   calving_trouble

    ⊙ Yes

    ⊙ No

    ⊙ Don't know

Is YES above

Do you know why you are having this problem?   calving_trouble_reason

    ⊙ Yes

    ⊙ No

    ⊙ Don't know

Do you observe rodents in or around the cattle house?

    ⊙ Yes   rodents

    ⊙ No

If YES above

Do you use any rodent control?   rodent_control

SECTION 4: ANIMAL BIODATA

Genotyped animal

Picture ear tag number   Photo   ear_tag

Animal ID number

    ⊙Select from listed ID/type   Enter last 4   id

Animal Age		animalage
Integer		

Animal breed		
⊙ SHZ-X-Friesian	Mark	appropriate breed
⊙ SHZ-X-Ayrshire	response	
⊙ SHZ-X-Jersey		
⊙ Other		

Dentition score		
⊙ 0	Mark	appropriate dentition
⊙ 1	response	
⊙ 3		
⊙ 4		

Body condition score		bcs
⊙ 1	Mark	appropriate
⊙ 2	response	
⊙ 3		
⊙ 4		
⊙ 5		

Animal Sex		animalsex
⊙ Male	Mark	appropriate
⊙ Female	response	

If female

Which option best describes this cow?

⊙ Heifer	Mark	appropriate
⊙ Cow with 1 or more calves	response	

If Heifer

When was she last served		service
⊙ Never	Mark	appropriate
⊙ Month/year	response	

If female had 1 or more calves

How many calves has this cow given birth to alive	calf_number

When did she last calve?		calf_date
Integer	Month/year	

Which option best describes the last calf?		calf_status
⊙ Normal healthy	Mark	appropriate
⊙ Borne weak but survived	response	
⊙ Born weak and died within first month		
⊙ Don't know		

Which option best describes getting the cow back in calf after the last calving?		calving_status
⊙ Not yet put to the bull	Mark	appropriate
⊙ Put to the bull but not pregnant	response	
⊙ Put to the bull and pregnant		

Which option best describes her current pregnancy status? — pregnancy_status

⊙ Don't know   Mark appropriate

⊙ Inseminated but not sure if pregnant   response

⊙ Pregnancy tested positive

⊙ Pregnancy tested negative

Has this cow ever aborted/premature dead calf? — abortion_status

⊙ Yes   Mark appropriate

⊙ No   response

⊙ Don't know

If YES above

When did she abort/have premature calf — abortion_date

Month/year

Genital discharge — genital_discharge

⊙ No genital discharge   Mark appropriate

⊙ Serous; Mucoid   response

⊙ Puruent; Bloody

⊙ Other; Not Evaluated

Udder condition — udder_status

⊙ Normal   Mark appropriate

⊙ Mastitic   response

⊙ Flabby

⊙ Other

Milk consistency   milk_status

    ⊙ Normal   Mark   appropriate

    ⊙ Bloody   response

    ⊙ Mucoid

    ⊙ Purulent

    ⊙ Other

Does the animal appear to be   Mark   appropriate   salivation

drooling   response

    ⊙ Yes

    ⊙ No

    ⊙ Not evaluated

Does the animal appear lame or   lameness

unwilling to move

    ⊙ Yes   Mark   appropriate

    ⊙ No   response

    ⊙ Not evaluated

FMD-like lesions   FMD_lesions

    ⊙ Mouth

    ⊙ Feet

    ⊙ Mouth and feet

    ⊙ None

    ⊙ Not evaluated

SECTION 5: ANIMAL SAMPLING

Sample collection   Mark   appropriate

               response

    ⊙ Yes

    ⊙ No

Serum Sample   serum_code

    Serum Sample Barcode   Scan bar code and hand
write 4 digit animal ID
and date on tube

EDTA Sample   EDTA_code

    EDTA Sample Barcode   Scan bar code and hand
write 4 digit animal ID
and date on tube

Vagina swab Sample   swab_code

    Vaginal   Swab   Sample
Barcode

Please estimate distance to next   distance
dairy farm

    ⊙ Less than 100m

    ⊙ 100-500m

    ⊙ More than 500m

GPS northing

GPS easting

RESEARCH OUTPUTS

(i)   Research Papers

Motto, S. K., Shirima, G. M., de Clare Bronsvoort, B. M., & Cook, E. A. J. (2021). Epidemiology of leptospirosis in Tanzania: A review of the current status, serogroup diversity and reservoirs. *PLoS Neglected Tropical Diseases*, *15*(11), e0009918.

Motto, S. K., Hernandez-Castro, L. E., Shirima, G. M., Mengele, I. J., Bwatota, S. F., Bronsvoort, B. M. D. C., & Cook, E. A. J. (2023). Seroepidemiology of *Leptospira* serovar Hardjo and associated risk factors in smallholder dairy cattle in Tanzania. *PLOS Neglected Tropical Diseases*, *17*(4), e0011199.
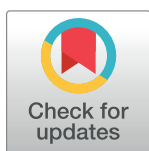
(ii)   Poster Presentation

RESEARCH ARTICLE

Epidemiology of leptospirosis in Tanzania: A review of the current status, serogroup diversity and reservoirs

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Abstract

Background

Tanzania is among the tropical countries of Sub-Saharan Africa with the environmental conditions favorable for transmission of *Leptospira*. Leptospirosis is a neglected zoonotic disease, and although there are several published reports from Tanzania, the epidemiology, genetic diversity of *Leptospira* and its host range are poorly understood.

Methods

We conducted a comprehensive review of human and animal leptospirosis within the 26 regions of the Tanzanian mainland. Literature searches for the review were conducted in PubMed and Google Scholar. We further manually identified studies from reference lists among retrieved studies from the preliminary search.

Results

We identified thirty-four studies describing leptospirosis in humans (n = 16), animals (n = 14) and in both (n = 4). The number of studies varied significantly across regions. Most of the studies were conducted in Morogoro (n = 16) followed by Kilimanjaro (n = 9) and Tanga (n = 5). There were a range of study designs with cross-sectional prevalence studies (n = 18), studies on leptospirosis in febrile patients (n = 13), a case control study in cattle (n = 1) and studies identifying novel serovars (n = 2). The most utilized diagnostic tool was the microscopic agglutination test (MAT) which detected antibodies to 17 *Leptospira* serogroups in humans and animals. The *Leptospira* serogroups with the most diverse hosts were Icterohaemorrhagiae (n = 11), Grippotyphosa (n = 10), Sejroe (n = 10), Pomona (n = 9) and Ballum (n = 8). The reported prevalence of *Leptospira* antibodies in humans ranged from 0.3–

29.9% and risk factors were associated with occupational animal contact. Many potential reservoir hosts were identified with the most common being rodents and cattle.

Conclusion

Leptospirosis is prevalent in humans and animals in Tanzania, although there is regional and host variation in the reports. Many regions do not have information about the disease in either humans or their animal reservoirs. More studies are required to understand human leptospirosis determinants and the role of livestock in leptospirosis transmission to humans for the development of appropriate control strategies.

Author summary

Bacteria from the genus *Leptospira* is an important agent for causing a disease called leptospirosis in humans and a range of animal species. Leptospirosis is often under-recognized as it presents varied symptoms that mimic malaria, typhoid, brucellosis and other diseases. More than 250 pathogenic *Leptospira* serovars are known to cause leptospirosis in humans and animals. The diversity of *Leptospira* serovars and their distribution in humans and animals is little defined in Tanzania. We conducted a systematic review to gather information on the diversity of *Leptospira* serovars with their reservoir distribution and the most common diagnostics methods used. We included studies (n = 34) in the review and found 17 serogroups described in 28 studies that utilized microscopic agglutination test (MAT). So far human and other animal hosts including cattle, dogs, pigs, bats, buffalo, fish, rodents, goats, lion, zebra, sheep and shrews have been investigated for leptospirosis in Tanzania. Our results show that cattle and rodents are likely to be important reservoirs of pathogenic *Leptospira* spp. and can be a source of human leptospirosis principally in the farming system. Further studies are needed to explore predominant serovars in livestock for the development of prevention strategies to reduce transmission and risks in humans.

Introduction

Leptospirosis is a serious infectious disease caused by spirochete bacteria in the genus *Leptospira* [1]. It is considered a re-emerging zoonosis widespread in tropical and sub-tropical regions, where there are limited surveillance and disease control measures [2]. Leptospirosis infections may be acute, subacute or chronic [1] and may result in severe health problems such as pulmonary haemorrhagic syndrome (PHS) [3], or renal and liver dysfunctions [2,4,5]. Leptospirosis often presents with varied symptoms that mimic those of several other unrelated febrile illnesses including dengue and malaria [6]. Therefore, leptospirosis is an important undifferentiated febrile illness that requires differential diagnosis [7].

The incidence of leptospirosis is poorly known and this may be partially attributed to inadequate data and surveillance [8]. In addition, there is a shortage of appropriate diagnostic facilities in developing countries, and clinicians may fail to recognize leptospirosis in febrile patients, consequently it remains underreported [2]. However, it is estimated that around the globe there are 1.03 million leptospirosis cases annually and 2.9 million Disability Adjusted Life Years (DALYs), where the majority of infections and burden are in low and middle-income countries (LMICs) [4,9].

Leptospires are mainly harboured in the renal tubule and excreted in the urine of accidental and maintenance hosts including cattle, rodents, pigs, dogs, sheep and goats [1,10]. Humans contract leptospirosis from contaminated environments, consumption or handling waste products from infected animals [1,11]. More than 250 serovars have been serotyped into 31 serogroups which can potentially cause leptospirosis in humans and animals worldwide [12,13]. Based on DNA hybridization techniques and phylogenetic analysis, 64 species have been recognized and rearranged into two clades (pathogenic "P" and saprophytic "S") and two subclades in each clade (subclade P1 and P2 and subclades S1 and S2) [14]. There are 17 species classified in subclade P1 of which 8 can cause severe disease in humans and 21 species in subclade P2 that can cause mild disease, and the remaining species, considered non-pathogenic, are in clade S subclade S1 and S2 [14].

Leptospirosis in Tanzania was reported in the early 1990s [15]. The authors of that study aimed to determine seroprevalence in humans, domestic and wild animals based on the microscopic agglutination test (MAT). The seroprevalence of *Leptospira* antibodies was reported as 38% in dogs, 5.6% in cattle, 1.8% in rodents and 0.3% in humans [15]. Despite the low prevalence of *Leptospira* antibodies in humans, it was sufficient to indicate a public health concern and the need for control and prevention strategies. Several studies have been conducted since and leptospirosis has been reported in a range of species [11,16–18]. Two recent investigations estimated human leptospirosis incidence in Tanzania. The study populations involved were hospitalized patients with fever related symptoms. The disease incidence was estimated by the two studies to be 75-102/100,000 persons annually in 2007–2008 [19] and 11-18/100,000 persons annually in 2012–2014 [20]. Humans are at high risk of contracting leptospirosis based on the fact that multiple animal species harbour and transmit the disease including livestock and wildlife [11,18,21]. Although three decades have elapsed since the first detection of leptospirosis in Tanzania the epidemiology and the diversity of leptospiral serovars and their reservoirs are not well articulated. This review comprehensively examined the disease epidemiology and *Leptospira* diversity in Tanzania to inform stakeholders of any existing knowledge gaps and for appropriate management of the disease.

Methods

Search strategy

A thorough and comprehensive search of the literature was carried out to identify studies associated with human, domestic or wild animal leptospirosis and *Leptospira* in Tanzania. To retrieve all related information, a boolean operator ("OR" and "AND") with a combination of keywords was set and both PubMed and Google Scholar electronic search engines were used to retrieve published papers, peer-reviewed articles, theses, case reports, posters and conference presentations. Retrieval of materials from PubMed and Google search engine was done on 24th May 2020. In the PubMed search engine search terms were: ('human' OR 'people' OR 'domestic animals' OR 'bovine' OR 'cattle' OR 'pigs' OR 'porcine' OR 'rodent' OR 'rat' OR 'dogs' OR 'canine' OR wildlife' OR 'wild animals') AND ("leptospirosis" OR '*Leptospira*' OR 'Weils disease' OR 'Weils syndrome' OR '*Leptospira* serovars' OR 'sokoine serovar' OR 'interrogans serovar' OR 'Icterohaemorrhagiae serovar' OR 'Hebdomadis serovar') AND ('Tanzania' OR 'Northern zone' OR 'Kilimanjaro' OR 'Morogoro' OR 'Rukwa' OR 'Katavi' OR 'Tanga' OR 'Kagera' OR 'Simiyu' OR 'Mara' OR 'Geita' OR 'Shinyanga' OR 'Songwe' OR 'Moshi') AND ('prevalence' OR 'epidemiology' OR 'risk factors' OR 'febrile illness' OR 'acute leptospirosis'); while in Google scholar (('*Leptospira*' OR 'leptospirosis') AND Tanzania)) were the key search terms used.

Study selection

The search returned a large number of publications, and the contents were collated in Mendeley citation manager version 1.19.4. Additional papers were identified from reference lists of retrieved articles to find appropriate studies that might not have been identified during the preliminary search. All papers were checked for duplicates and removed in Mendeley software. In the subsequent stage, those papers remaining after cleaning were then screened dependent on their titles and relevant geographical study location. Consequently, the full content of those papers was further assessed as far as their significance and by considering the inclusion and exclusion criteria.

Criteria for study eligibility

Inclusion and exclusion criteria. In this review, all publications including published papers, theses, poster or conference presentations were included if the source contained primary data citing leptospirosis/ febrile illness in humans, domestic or wild animals. Theses and poster presentations were excluded if the data had been published in another peer-review journal. All texts written in English and focused on Tanzania as the geographical area of attention were eligible.

Results

At a preliminary search, a total of 3767 documents were retrieved from two database search engines and pooled into the Mendeley citation manager. Of those articles, 3720 were recovered from Google Scholar and 47 from PubMed. A further 13 papers were searched and added manually after being identified from reference lists among the retrieved articles to make 3780 papers in total. Then articles were checked for duplicates in Mendeley, 3465 articles remained and met the criteria for the initial stage of inclusion and exclusion after duplicate removal. The initial screening was based on the title of the article and relevant study location (i.e. Tanzania), 3395 articles were excluded in the review process due to failure to fulfil the inclusion criteria for the next stage of assessment. A large number of articles recovered from Google scholar were excluded as they did not report leptospirosis in Tanzania. These articles were detected by the search engine because Tanzania was mentioned in the text of the paper as the author had referenced a previous publication. The publications were most often reporting leptospirosis in another country. After the selected literature underwent full text screening, 32 published papers were identified with primary data describing *Leptospira* and leptospirosis from Tanzania in humans and various animal species. In addition, two papers were identified, which were published after the initial retrieval was conducted, and these have been included in the review [22,23]. The flow diagram Fig 1 describes the process of identifying studies for this review. A summary of each study is available in S1 Table including the year of research, study design, geographical location, target populations, diagnostics tests, and results for each study (n = 34).

Of the 34 studies identified, sixteen described *Leptospira* seropositivity or leptospirosis in humans, fourteen investigated animals and four focused on both humans and animals S1 Table. There was a range of study designs with more than fifty per cent of studies being prevalence studies (n = 18). Over thirty percent were targeted studies investigating *Leptospira* as a cause of illness in febrile patients (n = 13) or disease in animals (n = 1) and a small number identified novel serovars (n = 2).

Geographic distribution of *Leptospira* studies

The Tanzanian mainland comprises 26 regions that are divided into 6 zones which are as follows: Lake Zone (Mwanza, Kagera, Shinyanga, Geita, Mara and Simiyu), Western Zone
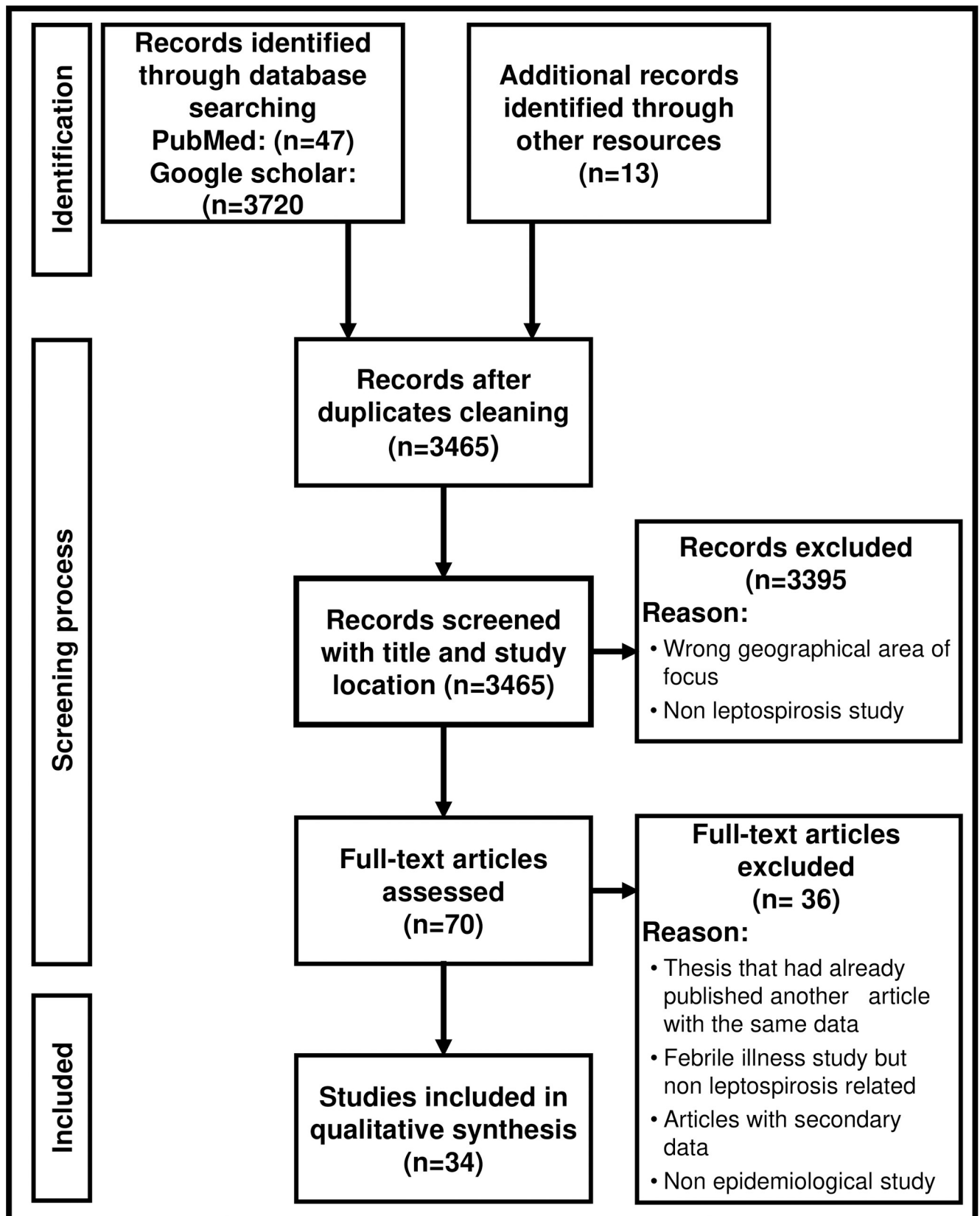
Fig 1. Flow diagram indicating how articles were included in the review regarding leptospirosis in Tanzania.
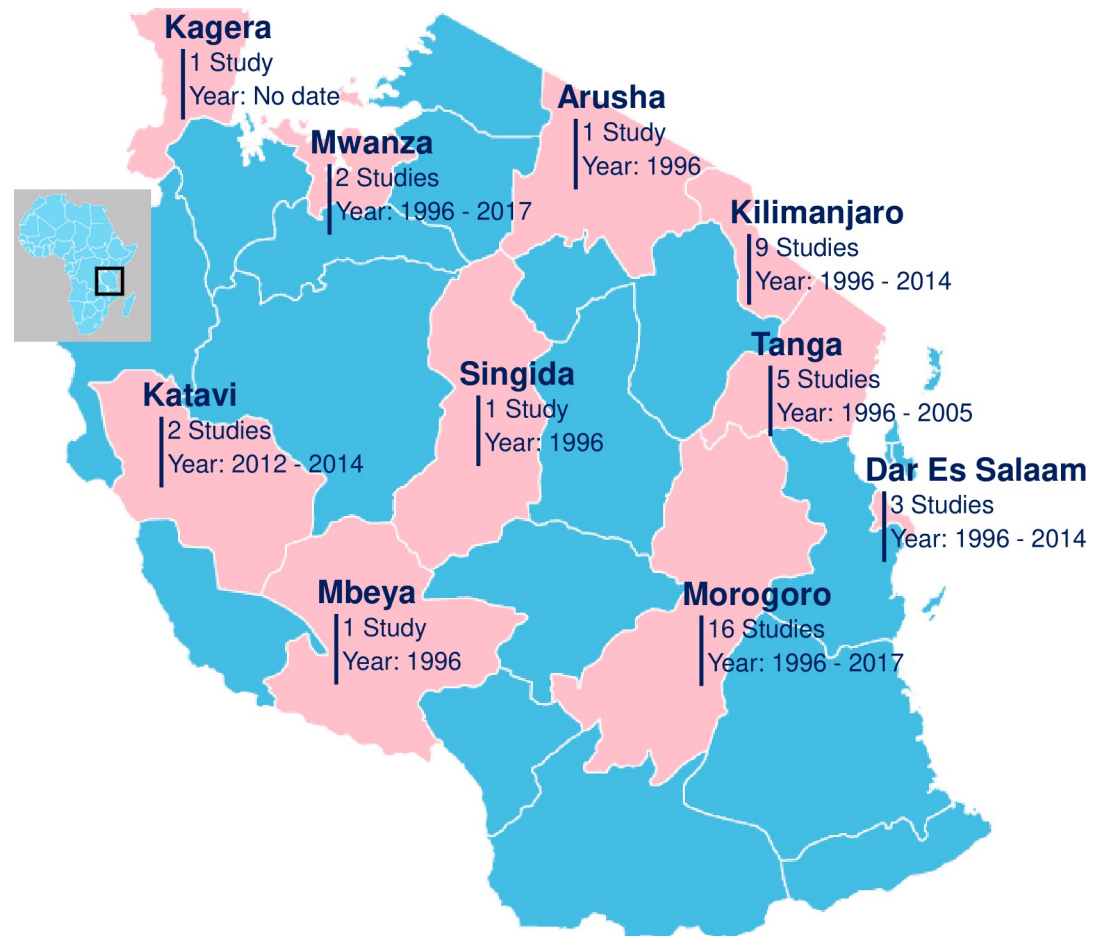
Fig 2. Geographical distribution of *Leptospira* studies reported from human, domestic and wild animals: Regions colored pink indicate areas with *Leptospira* studies from 1990s to date and regions colored blue indicate regions where no study was retrieved from the search engine. This map was prepared using Simplemaps https://simplemaps.com/resources/svg-tz.

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(Katavi and Kigoma), Southern Highland Zone (Songwe, Rukwa, Ruvuma, Mbeya, Iringa and Njombe), Eastern Zone (Morogoro, Pwani, Dar es Salaam, Lindi and Mtwara), Central Zone (Dodoma, Singida and Tabora) and Northern Zone (Kilimanjaro, Manyara, Tanga and Arusha). The geographical distributions of the recovered studies are shown in Fig 2.

There was an unequal distribution in the *Leptospira* studies conducted across the country. Human or animal related studies were only conducted in 10 (38.5%) out of 26 regions of the Tanzanian mainland. The majority of the studies reporting *Leptospira* or leptospirosis were from Morogoro region (n = 16) followed by Kilimanjaro (n = 9) and Tanga (n = 5). Additional studies were conducted in Dar es Salaam (n = 3), Katavi (n = 2), Mwanza regions (n = 2), Kagera (n = 1), Arusha (n = 1), Singida (n = 1) and Mbeya (n = 1) Fig 2. Only one study was conducted in multiple regions [15]. In some regions such as Mbeya and Singida the research was conducted many years ago at the onset of the disease identification in the country.

Studies from Morogoro region (n = 16) were mostly cross-sectional studies in animals (n = 8), or humans and animals (n = 2) and among these the animals studied were: rodents, shrews, cattle, goats, sheep, pigs, dogs, cats, fish and bats. The other studies from Morogoro described leptospirosis in hospital patients (n = 4) or new serovars (n = 2). On the other hand, studies conducted in the Kilimanjaro (n = 9) region were hospital-based studies describing

leptospirosis in humans (n = 7). There was one cross-sectional study in humans and animals (n = 1) and one study focused only on animals with the target animals being cattle, goats, sheep and rodents. Among the five studies in Tanga, there were four cross-sectional studies, including two animal studies, one human study, one study in both humans and animals, and one targeted study investigated clinical disease in animals. Of the studies in Dar es Salaam two were hospital based and one was a cross sectional study of animals and humans. The study in Arusha was hospital based and the studies in the remaining regions were cross sectional in humans (Katavi and Mwanza) and in both humans and animals (Kagera, Mbeya, Katavi, Mwanza and Singida).

Diagnostic approaches for detecting *Leptospira* or antibodies to *Leptospira*

Various diagnostic methods for leptospirosis were identified during the review S1 Table. These diagnostic techniques include microscopic agglutination test (MAT) (n = 28), culture and isolation (n = 7), cross agglutinin absorption test (CAAT)(n = 2), Eiken latex agglutination test (n = 1), enzyme linked immunosorbent assay (ELISA) (n = 1) and polymerase chain reaction (PCR) (n = 9). Despite the advancement of diagnostic technology, currently few studies use molecular typing [10,22,24] for characterising *Leptospira* sp. Most of the studies (n = 22) employed a single technique for leptospirosis detection. Microscopic agglutination test (MAT) was broadly utilized in 85% of the studies (n = 28) for leptospirosis diagnosis and in nine of these studies it was utilized in combination with other methods such as ELISA, culture, or PCR S1 Table. Recent studies used advanced diagnostics methods including either polymerase chain reaction, molecular typing or in combination (n = 9). For PCR, the studies used a variety of tissues such as kidney, culture isolate and blood sample for detection and the assays had different gene targets [10,22,23,25,26].

Leptospiral serogroups used in studies that utilized MAT

The studies utilizing the MAT test for detection of antibodies to *Leptospira* included a wide range of *Leptospira* serogroups Fig 3. In general, human studies tended to use a wider range of serogroups compared to studies from animals Fig 3 [20,27–29]. Serogroups commonly used in human studies included: Australis (n = 7), Ballum (n = 9), Canicola (n = 4), Grippotyphosa (n = 9), Hebdomadis (n = 7), Icterohaemorrhagiae (n = 10), Pomona (n = 6), Sejroe (n = 7), and Tarassovi (n = 4). *Leptospira* serogroup panels which have been widely used for animal studies have included: Australis (n = 7), Ballum (n = 12), Canicola (n = 7), Grippotyphosa (n = 7), Hebdomadis (n = 8), Icterohaemorrhagiae (n = 14), Pomona (n = 12) and Sejroe (n = 9). The serogroups investigated for each animal group were not always detected as indicated in Fig 3 and S1 Table.

Predominant *Leptospira* serogroups detected in human and animals

Thirty (n = 30) studies were able to report and describe serogroup diversity out of those studies using MAT, CAAT and molecular typing diagnostic approaches. The review found 17 *Leptospira* serogroups reported from humans and across animal species in Tanzania. In the case of humans, the most detected serogroups were Icterohaemorrhagiae (n = 11), Grippotyphosa (n = 8), Australis (n = 8), Ballum (n = 7), Hebdomadis (n = 6) and Sejroe (n = 6). We only counted the MAT serogroup once for samples that were used by multiple studies [19–21,27,30]. The most prevalent serogroups in people were Sejroe, Icterohaemorrhagiae and Australis Tables 1 and S2. The serogroups detected in the highest proportion of hospital patients were Australis, Icterhaemorrhagiae and Djasiman Tables 1 and S2.
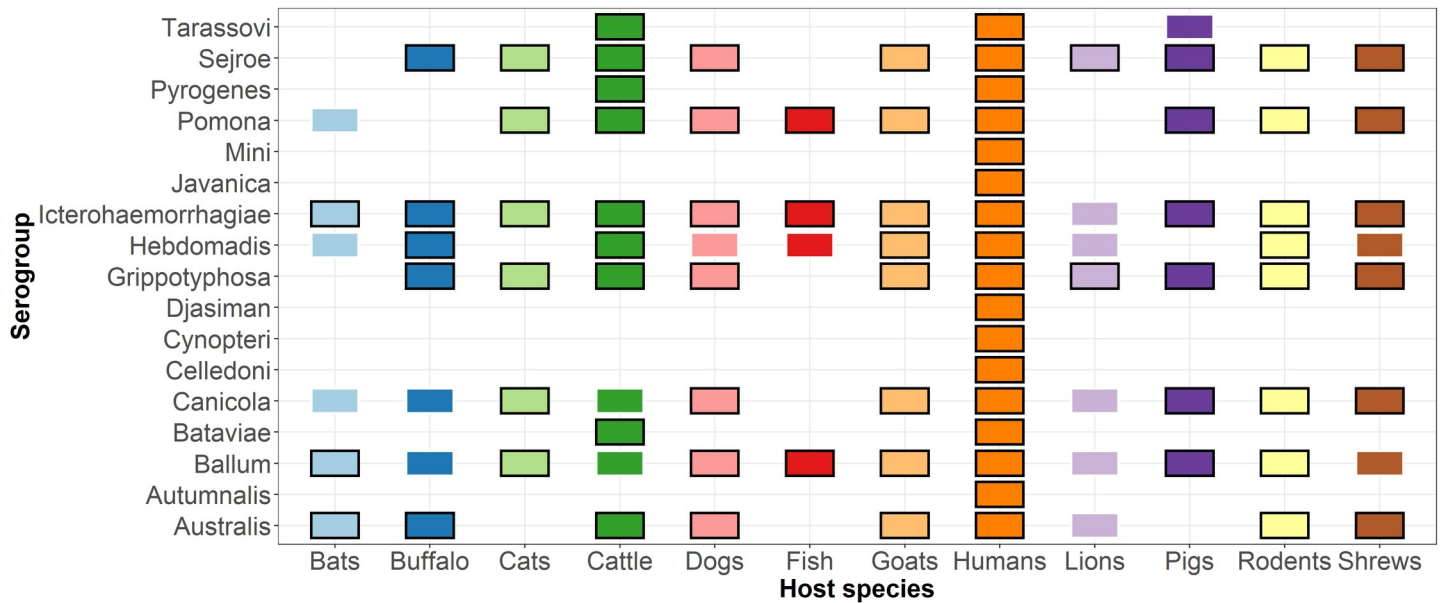
Fig 3. Serogroups used in the Microscopic Agglutination Test (MAT) for detection of antibodies to *Leptospira* in humans and animals in Tanzania (1997–2019). The coloured box indicates that samples were screened for these serogroups and the black outline indicates that the serogroup was detected (n = 28).

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The most predominant *Leptospira* serogroups being reported in different animals were Icterohaemorrhagiae in 11 different animals (cattle, rodents, shrew, dogs, goat, sheep, bats, buffalo, pigs, cats and fish), Grippotyphosa and Sejroe in 10 animals (cattle, rodents, shrew, dogs, goat, sheep, buffalo, lion, cats, pigs), Pomona in 9 animals (cattle, rodents, shrew, dogs, goat, sheep, pigs, cats and fish), and Ballum in 8 animals (rodents, dogs, goats, sheep, bats, pigs, cats, and fish). The study carried out in wildlife found zero leptospiral antibodies in zebras which may be due to the small number of samples tested [11]. The most prevalent serogroups in rodents were Australis and Icterohaemorrhagiae and in cattle the most prevalent serogroups were Sejroe and Tarassovi Table 1.

Leptospirosis and prevalence in humans

Leptospirosis in humans was reported by 20 eligible studies from 10 regions of Tanzania. Six of these studies were cross sectional studies investigating seroprevalence in the general population Table 2. The findings from two papers which conducted studies on people in Katavi from

Table 1. Mean prevalence of antibodies to *Leptospira* serogroups in people in cross-sectional studies; in febrile patients; in rodents; in cattle in Tanzania in leptospirosis papers published 1997–2021.

Study type, number and references	Seroprevalence (%)							
	Australis	Ballum	Djasiman	Grippotyphosa	Hebdomadis	Icterohaemorrhagiae	Sejroe	Tarassovi
Cross-sectional studies in people (n = 5) [15,26,31–33]	3.43	0.48	NT	1.58	1.50	5.38	9.35	1.00
Hospital based studies in febrile patients (n = 4) [21,27,29,34]	20.48	5.78	16.20	8.20	6.13	17.45	4.18	7.4
Cross sectional studies in rodents (n = 7) [11,15,31,35–38]	8.38	1.47	NT	2.07	0.28	7.29	0.37	NT
Cross sectional studies in cattle (n = 6) [11,15,39–41]	0.80	0.00	NT	4.80	5.10	4.25	15.94	15.10

NT—Not tested

https://doi.org/10.1371/journal.pntd.0009918.t001

Table 2. Summary of studies reporting leptospirosis and seroprevalence of antibodies to *Leptospira* in humans in Tanzania 1997–2019.

Reference	Year	Study area	N	Seroprevalence (%)	Acute leptospirosis (%)	Risk factors/exposure
[15]	1996	Morogoro, Dar es Salaam, Mbeya, Kilimanjaro, Tanga, Singida and Mwanza	375	0.3	ND	ND
[33]	2005	Tanga	199	15.1	ND	ND
[11]* [26]	2012–2013	Katavi	267	29.96	ND	Slaughtering and handling of bush meat
[32]	2017	Mwanza	250	10	ND	Abattoir workers and meat vendors
[31]	No date	Kagera	455	15.8	ND	Fishing and working in sugarcane plantation
[18]	1996–2006	Morogoro	506	ND	0.2 Patients	ND
			83	ND	3.6 Abattoir workers	ND
[30]	2007–2008	Kilimanjaro	831	ND	8.4	ND
[42]	2008	Dar es Salaam	1005	ND	0.47	ND
[34]	2013	Morogoro	370	ND	11.6	Heavy rain and presence of rodents in residential areas
[43]	2014	Morogoro	191	ND	2	ND
[23]	2013–2014	Dar es Salaam	519	ND	0.2	ND
[25]	2014	Morogoro	842	ND	3	ND
[21]	2012–2014	Kilimanjaro	1293	ND	1.9	Cleaning animal waste and rice farming
[29]	2016–2017	Arusha	104	ND	5.8	ND

ND: not described

*Studies used the same data

2012–2014 are reported once [11,26]. From 1997–2019, a total of 209 out of 1546 tested samples were seropositive for antibodies against *Leptospira* spp. serogroups Table 2. The prevalence of antibodies to *Leptospira* varied depending on the study area, study design and interpretation of the results from 0.3% to 29.9%. Risk factors identified from 5 studies include occupational exposures such as contact with animals, animal waste and animal products Table 2.

There were also 14 hospital-based studies examining acute leptospirosis. Seven papers reported findings from the same patients in Kilimanjaro from 2007–2008 and/or 2013–2014 [10,19–21,27,28,30]. We have only reported the acute cases from these seven studies as defined by the authors and reported in 2 papers [21,27]. From 1997–2019 there were 173 acute cases of leptospirosis identified from 5661 febrile patients. Additionally, one study reported *Leptospira* in the urine of abattoir workers (3/83) which is not included in this number [18].

Leptospirosis incidence was estimated by two systematic hospital based and health care utilization surveys from the Kilimanjaro region. There was a large difference in the incidence estimations between the two studies. One study was conducted between 2007–2008 with the calculated incidence of acute leptospirosis ranging from 75–102 per 100,000 people annually [19]. The other study reported a lower leptospirosis incidence of 11–18 cases per 100,000 people annually from 2012–2014 [20].

Animal leptospirosis and prevalence

Several leptospirosis studies have been carried out in various animal species in Tanzania, and in this review, a total of 18 studies met the inclusion criteria and were examined, 15 were cross sectional prevalence studies Table 3, 1 case control study [39] and 2 identified new serovars [44,45]. The total number of animals tested was 9090, though there were variations in the sample size and species between regions. The animals investigated were rodents (n = 10), shrews (n = 7), cattle (n = 8), goats (n = 3), pigs (n = 2), dogs (n = 2), bats (n = 2), sheep (n = 1), fish (n = 1), buffaloes (n = 1), lions (n = 1), zebra (n = 1). Eleven animal types were confirmed to have been exposed to *Leptospira*. These include rodents, shrews, cattle, goats, pigs, dogs, bats, sheep, fish, buffaloes, and lions Table 3. Among the animals studied, rodents (*Aesthomys chrysophilus*, *Dasmys incomtus*, *Mastomys natalensis*, *Rattus rattus*, *Lemniscomys griselda*, *Lemniscomys rosalia* and *Gerbilliscus vicinus*) were the most investigated followed by cattle in Tanzania. The prevalence of antibodies to *Leptospira* in cattle ranged from 5.6–51.0%, and in rodents from 1.8–25.8%. The presence of antibodies in serum samples was determined by MAT with recent studies adopting qPCR and molecular sequencing to confirm the infection from kidney samples for explorations of *Leptospira* serogroups diversity [10,22].

Discussion

This review gives an insight on *Leptospira* prevalence and exposure, leptospirosis and the predominant *Leptospira* serogroups and their diversity in human and animal populations in Tanzania. It is evident after a detailed review of the published literature that leptospirosis is a prevalent zoonosis in Tanzania and present in various hosts including humans, livestock, wild animals, and aquatic life. There is an uneven distribution of research studies with large regions having inadequate or no leptospirosis information. The presence of universities or research institutions in regions that were overrepresented may reflect a degree of bias in the study site selection. For example, Kilimanjaro Clinical Research Institute (KCRI) conducted several human leptospirosis studies in the northern part of Tanzania while the Sokoine University of Agriculture conducted predominantly animal studies in the Morogoro region.

Our findings show that human leptospirosis is an important zoonosis of public health impact in Tanzania. Leptospirosis is widespread and prevalence varies between different settings and different populations. The actual burden of leptospirosis in humans may be difficult to estimate due to the limited and uneven distribution of studies and disease underestimation in the country. However, this trend is not unique to Tanzania, with the majority of low and middle-income countries (LMICs) facing similar challenges of inadequate surveillance data and diagnostic facilities [2]. Similar reports of leptospirosis prevalence as identified in this review have been reported in neighbouring countries. A study conducted in Kenya reported an apparent seropositivity of 13.4% in slaughterhouse workers [48], and a study of non-pregnant women in Uganda found 35% seropositive [49].

There was a large difference between the incidence reported in 2007–2008 and 2012–2014 in Kilimanjaro. This may be due to differences in the population selected, sample size or there may be variation in the leptospirosis incidence dependant on unknown factors [19,20]. Human leptospirosis in Tanzania may result from complex interactions between humans, animal carriers (such as cattle, rodents, dogs and pigs), and environments that favour perpetuation of leptospires and disease transmission.

The serological approaches utilized by various studies identified a diversity of *Leptospira* serogroups circulating in humans and animals. The MAT test was used in the majority of studies. MAT is a widely used diagnostic reference method for many studies, though not accessible in many laboratories due to its cost. MAT testing has many limitations: high levels of

Table 3. Summary of studies reporting animals with leptospirosis in Tanzania 1997–2019.

Reference	Year	Study area	Animal species	N	Seroprevalence (%)	*Leptospira* detected by culture* or PCR** (%)
[15]	1996	Morogoro, Dar es salaam, Mbeya, Kilimanjaro, Tanga, Singida and Mwanza	Cattle	MAT n = 374	5.6	
			Cattle	Culture n = 1021		0.7*
			Dogs	208	38	
			Rodent	537	1.8	
[18]	1996–2006	Morogoro	Giant pouch rats	285		8.4*
			Field rats	1382		0.6*
			Shrews	298		3.7*
			Goats	100	38	
			Pigs	100	41	
			Dogs	100	39	
			Cats	64	14.1	
			Small rodents	500	5	
			Small rodents	90	16.9	
			African giant rats	65	15.4	
			Shrew	4	25	
[17]	2003	Morogoro	Fish	48	54.2	
[37]	No date	Morogoro	Rodent	20	0	0* & 5**
			Shrew	7	0	29* & 29**
[41]	2002–2004	Tanga	Cattle	51	51	
[40]	2003–2004	Tanga	Cattle	655	30.3	
[39]	2005	Tanga	Cattle	80	21.3	
[46]	2007–2008	Morogoro	Pig	MAT n = 385	4.4	
				Culture n = 236		0.8*
[36]	2007–2008	Morogoro	Rodent and shrew	348	17.8	
[11]	2012–2013	Katavi	Cattle	1103	30.37	
			Goat	248	8.47	
			Rodent	207	20.29	
			Shrew	11	9.09	
			Buffalo	38	28.95	
			Lion	2	50	
			Zebra	2	0	
[16]	2013	Morogoro	Bat	36	19.4	
[38]	2012–2013	Morogoro	Rodent	89	25.8	
			Shrew	1	100	
[10]	2013–2014	Kilimanjaro	Cattle	452		7**
			Goat	167		1.2**
			Sheep	89		1.1**
			Rodents	384		0**
[47]	2016–2017	Morogoro	Dogs	232	9.5	
[35]	No date	Morogoro	Rodents	70	22.9	

(Continued)

Table 3. (Continued)

Reference	Year	Study area	Animal species	N	Seroprevalence (%)	*Leptospira* detected by culture* or PCR** (%)
[31]	No date	Kagera	Shrew and rodent	24	16.7	0*

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detectable antibodies are needed for a positive result and usually do not occur before the fourth week after disease onset [50] and it is time consuming and labour intensive [51,52]. Despite these drawbacks, the MAT test remains the only gold standard serological test and is considered a reference diagnostic test for leptospirosis in many settings [6,53].

The review found a large variation in the serogroup panels and the definition of positivity used across the studies S1 Table. When establishing a diagnostic panel it is advisable to include locally circulating serogroups or if these are not known to include a wide panel of pathogenic serogroups [6]. A list of candidate *Leptospira* serovars for diagnosis of leptospirosis using MAT in the African region was recently published based on research conducted in Tanzania [18]. However, emergence of new serovars suggests widening the serovar panels [14].

Most serogroups detected in animal species in the reviewed studies were also reported in humans. The most prevalent serogroups detected in rodents were Australis and Icterohaemoraghiae and Sejroe in cattle. These were also the most prevalent serogroups detected in people. This suggests that rodents and cattle may be an important source of infection in these settings. However, it is difficult to demonstrate transmission between animals and humans in our review because of the variability in the serogroup panel and different study designs.

A variety of domestic and wild animals in eighteen studies provide evidence of leptospirosis infections in animal populations in Tanzania. The review suggests that the main animal reservoirs for human leptospirosis may vary across the country, with primarily cattle, rodents, pigs, and dogs playing significant roles in disease transmission to humans. Rodents are important reservoirs of pathogenic *Leptospira* in many settings [12]. This review identified 10 studies reporting evidence of *Leptospira* in rodents and a diverse range of serogroups were detected Fig 3. There were only two studies in which *Leptospira* was detected in the sampled rodents using culture and PCR [18,37]. The lack of evidence of *Leptospira* in rodents in other studies using culture and qPCR techniques may indicate a methodological problem or lack of infected animals [22]. This scenario has also been reported in other studies, though such studies were associated with a limited sample size [12]. There may be differences in the prevalence of *Leptospira* in rodents between regions and between rural and urban settings [54]. Inappropriate sampling technique, sample preservation and an inadequate number of micro-organisms or loss of bacteria during culture can lead to false negative results.

Among exposed animals, cattle had the highest seropositivity, though this varied depending on geographical area. Cattle may be potential reservoirs and sources of human infection in Tanzania, particularly in rural areas where the majority of residents are smallholder dairy farmers and pastoralists [55]. Cattle are an important maintenance host for serogroup Sejroe [1,56] and transmission to farm workers and slaughtermen has been documented [48,57]. Animal contact particularly occupational exposures was identified as a risk factor by the reviewed papers and this is likely to have an important role in the epidemiology of leptospirosis in people in Tanzania [11,21,31,32].

Conclusion and recommendation

This review provides a summary of important information on the prevalence and distribution of the predominant *Leptospira* serogroups in humans and animals in Tanzania. Our review

suggests that more comprehensive leptospirosis studies are needed in rodents and livestock across different agro-ecological zones for a deeper understanding of the epidemiology and to understand the risks of human leptospirosis for better management and control of the disease. The role of livestock in disease transmission among the smallholder farmers and other risk factors for human leptospirosis should be well studied for future disease control plans.

In most studies conducted in Tanzania, the MAT is the only diagnostic test used widely for leptospirosis detection however MAT may be impractical in many clinical laboratories due to the cost and complexity [52]. An alternative tool, such as rapid diagnostic tests (RDTs), was proposed by a recent policy brief and may be appropriate in a clinical setting for routine screening of patients with non-malaria fever [58]. The performance of RDTs is variable and would need to be trialled before implementation [28,59]. Raising awareness among health providers and the community on leptospirosis is recommended as a vital strategy for disease control and prevention.

Supporting information

S1 Table. Summary of the papers included in this review of leptospirosis in Tanzania 1997–2019 including year of research, study design, geographical location, target populations, diagnostics tests, and results for each study.
(DOCX)

S2 Table. A) Prevalence of antibodies to *Leptospira* serogroups in people in cross-sectional studies; B) in febrile patients; C) in rodents; D) in cattle in Tanzania in leptospirosis papers published 1997–2021.
(DOCX)

Acknowledgments

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Seroepidemiology of *Leptospira* serovar Hardjo and associated risk factors in smallholder dairy cattle in Tanzania

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Abstract

Background

Smallholder dairy farming is crucial for the Tanzanian dairy sector which generates income and employment for thousands of families. This is more evident in the northern and southern highland zones where dairy cattle and milk production are core economic activities. Here we estimated the seroprevalence of *Leptospira* serovar Hardjo and quantified potential risk factors associated with its exposure in smallholder dairy cattle in Tanzania.

Methods

From July 2019 to October 2020, a cross-sectional survey was carried out in a subset of 2071 smallholder dairy cattle. Information about animal husbandry and health management was collected from farmers, and blood was taken from this subset of cattle. Seroprevalence was estimated and mapped to visualize potential spatial hotspots. The association between a set of animal husbandry, health management and climate variables and ELISA binary results was explored using a mixed effects logistic regression model.

Results

An overall seroprevalence of 13.0% (95% CI 11.6–14.5%) for *Leptospira* serovar Hardjo was found in the study animals. There was marked regional variations with the highest seroprevalence in Iringa 30.2% (95% CI 25.1–35.7%) and Tanga 18.9% (95% CI 15.7–22.6) with odds ratios of OR = 8.13 (95% CI 4.23–15.63) and OR = 4.39 (95% CI 2.31–8.37), respectively. Multivariate analysis revealed the individual animal factors that were a significant risk for

Leptospira seropositivity in smallholder dairy cattle were: animals over 5 years of age (OR = 1.41, 95% CI 1.05–1.9); and indigenous breed (OR = 2.78, 95% CI 1.47–5.26) compared to crossbred animals SHZ-X-Friesian (OR = 1.48, 95% CI 0.99–2.21) and SHZ-X-Jersey (OR = 0.85, 95% CI 0.43–1.63). Farm management factors significantly associated with *Leptospira* seropositivity included: hiring or keeping a bull for raising purposes (OR = 1.91, 95% CI 1.34–2.71); distance between farms of more than 100 meters (OR = 1.75, 95% CI 1.16–2.64); cattle kept extensively (OR = 2.31, 95% CI 1.36–3.91); farms without cat for rodent control (OR = 1.87, 95% CI 1.16–3.02); farmers with livestock training (OR = 1.62, 95% CI 1.15–2.27). Temperature (OR = 1.63, 95% CI 1.18–2.26), and the interaction of higher temperature and precipitation (OR = 1.5, 95%CI 1.12–2.01) were also significant risk factors.

Conclusion

This study indicated seroprevalence of *Leptospira* serovar Hardjo, as well as the risk factors driving dairy cattle leptospirosis exposure in Tanzania. The study showed an overall high leptospirosis seroprevalence with regional variations, where Iringa and Tanga represented the highest seroprevalence and risk. The study highlighted the urgent need to understand the human exposures and risks from this important zoonosis to develop control measures and awareness of the problem and quantify the economic and production impacts through abortion and milk loss. In addition, given that the available data was limited to *Leptospira* serovar Hardjo, the study recommends more studies to identify serologically the most common serovars in cattle for targeted vaccination and risk reduction.

Author summary

Dairy production in Tanzania constitutes traditional cattle meat-milk, improved smallholder dairy and commercial dairy farms. Despite the slow growth of the sector, smallholder dairy farming system remained a crucial for income generation and employment for thousands of families. This is more evident in the northern and southern highland of Tanzania where over 70% improved dairy cattle and milk production are core economic activities. Although the proportion of improved dairy cattle is relatively small compared to indigenous cattle, improved dairy sector contributes to 30% of milk produced in Tanzania. Constrains of leptospirosis in dairy, particularly of serovar Hardjo, remain a problem of its ability to cause abortion and reduce milk production in many farms worldwide. For many years epidemiological surveillance of leptospirosis in Tanzanian dairy cattle population is limited. This study provides a current status of seroprevalence and driven risk factors of leptospirosis occurrence in smallholder dairy cattle population from six regions of Tanzania as well as mapping hotspot areas at the district administrative level.

Introduction

Leptospirosis is a zoonotic disease caused by different serovars of *Leptospira spp*. The annual global human morbidity measured as Disability Adjusted Life Years (DALYs) is estimated to be 1.3 million and annual mortality is 580,000 people [1]. As a result, leptospirosis has been declared a worldwide public health disaster with the highest prevalence in tropical and subtropical countries where cases increase mainly during the wet season [2–4]. People who work

in particular dairy farming systems can contract leptospires via skin cuts, abrasions and mucous membranes after exposure to contaminated urine, reproductive fluids, manure, mud or pasture [5]. While animals acquire infection through sharing pasture or water contaminated with urine from infected animals.

Currently, over 300 serovars have been identified, many of them are pathogenic in humans and animals [6–8]. The clinical presentation varies depending on animal immunity and serovar type with possible asymptomatic cases in livestock [9,10]. Specifically, *Leptospira* serovar Hardjo (*L. interrogans* and *L. borgpetersenii*) causes reproductive complications (stillbirth, abortion, infertility, and death) in cattle [11].

In Tanzania, leptospirosis is a major public health issue and many studies have reported seropositive cases or active *Leptospira* infections in humans, domestic and wild animals [12–16]. The earliest evidence of leptospirosis was documented in the late 1990s when *L. interrogans* serovar Hardjo was confirmed [14] for the first time in livestock as well as in people. *Leptospira* serovar Hardjo seropositivity of 15.0% has been reported both in traditional and smallholder dairy herds in Tanga [17], with an additional study showing 3% seropositivity for *Leptospira* serovar Hardjo in at risk occupational groups in the same region [18]. Similarly, a study conducted in Katavi region reported *Leptospira* serovar Hardjo seropositivity of 17.59% in cattle and 15.73% in humans [19].

The dairy production system in Tanzania consists of three sectors: traditional cow meat-milk, improved small-holder dairy and commercial dairy farms [20]. Although the proportion of improved dairy cattle is relatively small compared to indigenous cattle (2.5% of total cattle number), the improved dairy sector contributes to 30% of milk produced in Tanzania [21]. The southern highlands and northern part of Tanzania have about 70% of improved dairy cattle and are core milk-producing areas in the country [21]. Over 90% of these are grouped into smallholder dairy farmers settled across rural and peri-urban areas [22]. Previous work has indicated that more than 90% comprises smallholder dairy cattle farms keeping one to five cows, and practicing intensive farming system on 1–2 hectares in southern and northern part of Tanzania [21,23]. A recent review of leptospirosis epidemiology in Tanzania [24] demonstrated that surveillance of *Leptospira* serovars is lacking in many areas, particularly in dairy cattle. Despite the importance of *Leptospira* serovar Hardjo in livestock health and productivity as well as its potential to cause abortion, little effort has been made on investigating disease prevalence in dairy cattle and risks factors for exposure.

Materials and methods

Ethics statement

Ethics of the study for animal subjects was reviewed and approved by the International Livestock Research Institute Institutional Animal Care and Use Committee (ILRI-IACUC2018-27) and the research permit was granted by the Tanzania Commission for Science and Technology (COSTECH), Ref. (2019-207-NA-2019-95). Written consent forms were signed by cattle owners before the interview and sample collection. The qualified Livestock Field Officer (LFO) restrained the animals during sampling. Local approval was sought from regional and local government authorities (LGAs) under the Ministry of Livestock and Fisheries (MLF).

Area of study

Two key geographical zones (Fig 1a) representing 70% of the total improved dairy cattle across the country were chosen in this study [21]. The northern zone included the regions of Kilimanjaro, Arusha and Tanga (Fig 1b), whereas the southern highland zone was mainly formed by the Iringa, Njombe and Mbeya regions (Fig 1c).
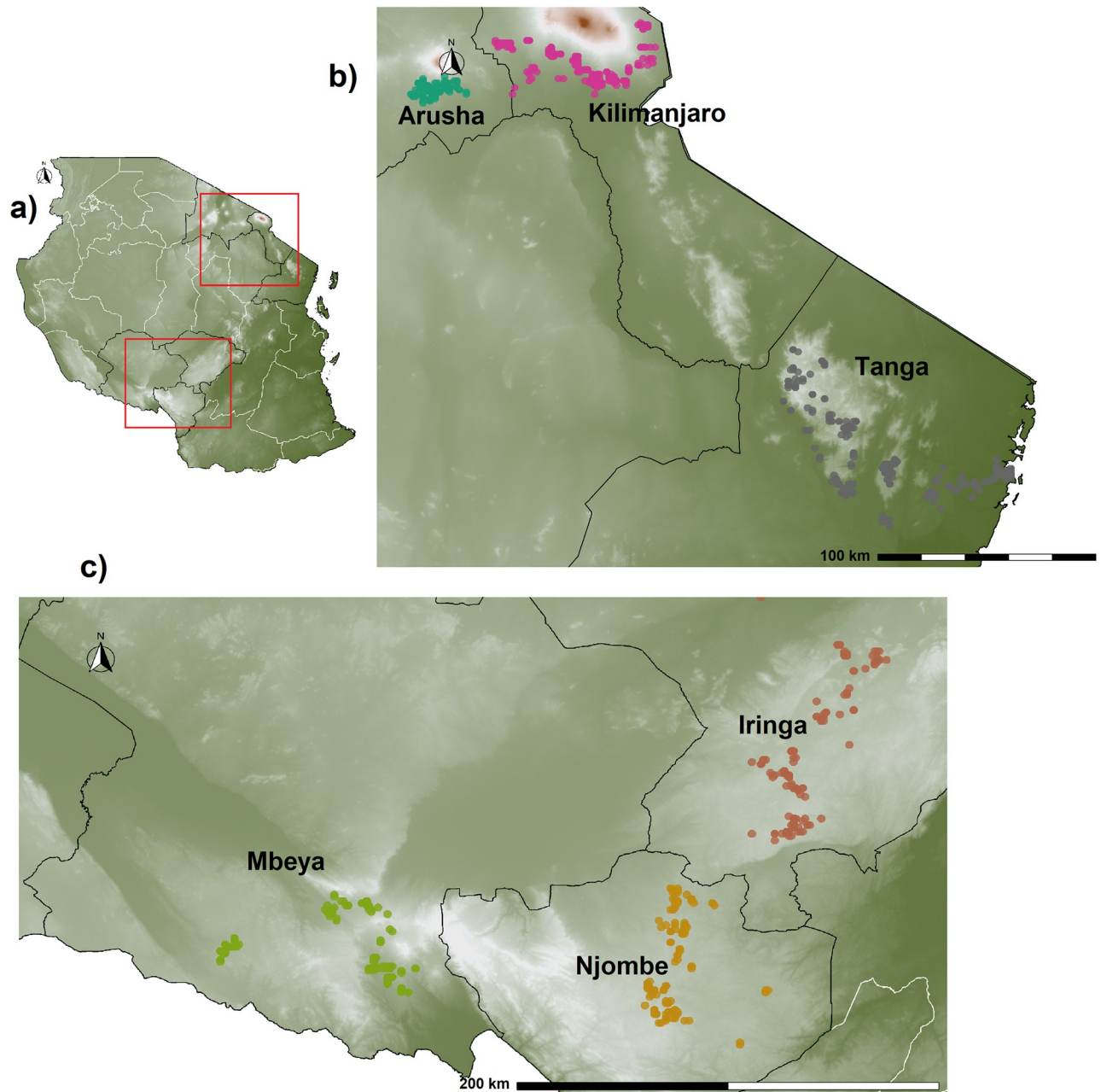
Fig 1. Geographic location of farms, regions, and dairy zones in Tanzania. a), geographic location across six regions from two economically important dairy zones over an elevation map of Tanzania. Red squares indicate the important dairy zones. **b)**, a close-up of the northern zone integrated by the regions of Arusha, Kilimanjaro and Tanga in which a total of 12 districts were sampled. **c)**, a close-up of the southern highland zone of Tanzania integrating the Iringa, Njombe, and Mbeya regions in which 11 districts were sampled. In all panels, farm location (dots) is colour-coded to indicate their administrative region. Map source: https://www.usgs.gov/centers/eros/science/usgs-eros-archive-digital-elevation-global-multi-resolution-terrain-elevation.

https://doi.org/10.1371/journal.pntd.0011199.g001

Study design

A cross-sectional study was carried out from July 2019 to October 2020. The cattle population in this study was selected from a subset of the cattle registry of the Africa Dairy Genetics Gains (ADGG) (https://data.ilri.org/portal/dataset/adgg-tanzania) program. Cattle (n = 50,000) had

previously been enrolled in the ADGG program and smallholder dairy farmers participated in monthly data collection activities related to animal production. Of these 4000 cattle had known genetic characteristics and could be identified by their preliminary information such as an ear tag number, age, and sex.

For possible leptospirosis risk factors in smallholder dairy farming, we designed a questionnaire survey which was uploaded to the Open Data Kit (ODK) cloud platform software (https://getodk.org) version 1.22.4, and accommodated in Android device. The farm owner or animal caretakers were interviewed, and their answers were recorded onto the ODK form. The information collected included demographic and herd management details, animal health data, vaccination practices, water sources, and presence of rodents, dogs, cats or pigs on the farm or neighbouring farms. Additionally, geographic coordinates of each farm were recorded to map the seropositive animals and farms after laboratory testing. Final forms were transferred via secure network connection, and aggregated on the server at ILRI, Nairobi, Kenya prior to analysis.

Serology sample

A blood sample was collected from the jugular vein into a 10ml blood collection tube (BD Vacutainer with no additives). Tubes were barcoded, labelled with date, animal identification number, and the barcode was scanned into the ODK survey form to link the animal biodata and the farm/herd owners. While in the field, samples were allowed to clot in a cool box filled with icepacks. Serum was prepared in the laboratory and stored at -20°C before testing as previously described [19].

Leptospira ELISA

The Linnodee *Leptospira* Hardjo ELISA Kit (Linnodee Animal Care, Oakmount, Holestone Road, Ballyclare, Northern Ireland BT39 0TJ) was used to test sera for the presence of antibodies against lipopolysaccharide (LPS) epitopes that are found on *Leptospira* serovar Hardjo envelope [11,25]. Test sera were added to a 96 well-plate along with positive and negative controls provided in the kit and the test run as previously described [11]. Finally, the optical density (OD) was measured at 450nm using the Synergy HTX Multi-Mode Microplate Reader (BioTek Instrument, Inc. Highland Park, Winooski, VT 05404–0998) and used to calculate the positivity ratio (PR).

$$PR = \frac{Mean\ sample\ OD - Mean\ negative\ control\ OD}{Mean\ positive\ control\ OD - Mean\ negative\ control\ OD}$$

The sensitivity and specificity of this ELISA have previously been reported to be 100% and 86.67%, respectively [26].

Statistical analyses

Seroprevalence estimates were calculated by dividing the number of positive samples by the number of cattle sampled. We also calculated an adjusted seroprevalence accounting for the stratified sampling design using *svydesign* functions in the *survey* R package [27]. Weights for each region were calculated by dividing the cattle population in each region by the number of sampled cattle [28].

We performed univariable analyses in the *epitools* R package [29] to measure associations at animal level, environmental and farm management variables (age, sex, breed, region, water source, herd size, abortion, multiple farm milking practices, hiring bull for breeding, presence of rodent in farm, grazing system, farm to farm distance, education and training by the farmer,

farmer's gender, experience in dairy farming, disposal of aborted/placental material, animal body condition score, animal contact with pigs and cat) and the binary ELISA results. Additional environmental data such as population density and solar radiation were sourced from the open.africa, elevation map on USGS, land cover on CCI Land Cover LC, and the mean annual temperature, precipitation from worldclim.org. To avoid multicollinearity, the Spearman's rank correlation coefficient (rho) and Pearson tests were run on continuous environmental variable pairs to ensure they were uncorrelated (rho < 0.29 based on Cohen [30].

All variables with significance ($p < 0.05$) association in univariable analyses and uncorrelated continuous variables were further considered for multivariable risk factors analyses. To model the relationship between our ELISA binomial results and a set of covariates, we built a binomial (logistic) generalised mixed effects model with a logit link function implemented in the template model builder *glmmTMB* package [29]. Model selection was a backward stepwise approach where all significant variables ($p < 0.05$) from univariable analysis and continuous environmental variables were included in the initial model and eliminated one at time. Nested models were compared using the Akaike Information Criterion (AIC) and those models with the lowest AIC were kept until the end. When two nested models had a very similar AIC, likelihood ratio tests allowed us to identify the best model (X^2 statistic $p < 0.05$; see S1 Table). Further, a final model was assessed by simulating residuals using the *simulateResiduals* function from the *DHARMa* package and estimating the amount of variance explained by the model (marginal and conditional R^2). The model was considered efficient if residuals were plotted versus fitted values and each fixed effect showed no clear pattern.

Results

Descriptive results

A total of 2086 out of 4000 animals were sampled from 1370 dairy farms. The reduced number of animals was due to animals being sold, slaughtered or having died. Of these 2086, 15 animals were excluded since they could not be linked to the main ADGG animal registry. The total number of animals sampled per region was Tanga (n = 523), Kilimanjaro (n = 520), Arusha (n = 318), Iringa (n = 305), Mbeya (n = 218), and Njombe (n = 187). The mean age of the sampled cattle was 5.5 years. Of the farms visited, the average animal per herd was 2 and animals were mostly (97.3%) clinically healthy females without udder or reproductive complications. Sampled animals were categorized into four breed types based on their records from the ADGG cattle registry. There were three crossbreed groups including crosses of shorthorn zebu (SHZ) with European breeds such as Friesian (SHZ-X-Friesian), Ayrshire (SHZ-X-Ayrshire) and Jersey (SHZ-X-Jersey), and the fourth group included all indigenous/local breeds. The highest number of animals were SHZ-X-Friesian (n = 1415), followed by SHZ-X-Ayrshire (n = 433), SHZ-X-Jersey (n = 144), and indigenous breed (n = 79). Over 80% of the farms were close to the neighbouring farm (within 100 meters) demonstrating intensive farming system with few herds practicing extensive pasture grazing system (distance between farms 100–500 meters). Our environmental data set showed a mean annual temperature and precipitation of 19.9°C and 1238mm, respectively; however slightly variations were present between regions. No farms reported vaccinating against *Leptospira* or any other preventative measures to *Leptospira* infection.

Seroprevalence

Of the 2071 animal sera tested, 269 (13.0%, 95% CI 11.6–14.5%) had antibodies against *Leptospira* serovar Hardjo. The adjusted seroprevalence accounting for the study design and differences in regional population sizes was 7.9% (95% CI: 3.9–11.8%).

The seropositivity was significantly related to breed with a high proportion of indigenous cattle being seropositive, 38.0% (95% CI 27.3–49.6%) compared to 12.7% (95% CI 11.0–14.6%) in SHZ-X-Friesian, 11.1% (95% CI 6.5–17.4%) in SHZ-X-Jersey, and 9.9% (95% CI 7.3–13.1%) in SHZ-X-Ayrshire (Table 1).

There was marked regional variation with the highest seroprevalence in Iringa Region 30.2% (95% CI 25.1–35.7%) and Tanga Region 18.9% (95% CI 15.7–22.6%).

The spatial distribution and leptospirosis hotspots in dairy cattle at the district administrative level in the six regions of northern and southern part of Tanzania are demonstrated in Fig 2. Briefly, in Iringa Region the following districts were identified as hotspots, Mufindi District Council, Iringa Municipal Council, and Mafinga Town Council, and in Tanga Region, Tanga Town Council, and Korogwe District Council were identified as hotspots for seropositive cattle.

Potential risk factors

The univariable analysis was performed with twenty-five variables at the initial screening. However, fourteen variables grouped at animal level and farm management were identified significantly associated to leptospirosis occurrence in Tanzanian dairy cattle ($p \leq 0.05$) which were included in multivariable analysis. Uncorrelated continuous environmental variables (temperature and precipitation) were also included in multivariable analysis.

The significant variables included animal level such as breed in which indigenous animals were significantly more likely to be seropositive than other breeds (OR = 5.55, 95% CI 3.19–9.65); male animals were more likely to be seropositive (OR = 2.74, 95% CI 1.56–4.80); animals aged over 5 years (OR = 1.83, 95% CI 1.41–2.37); and animals which had abortion in the previous 12 months (OR = 2.07, 95% CI 1.43–2.99).

Management factors that were significantly associated with leptospirosis seropositivity after univariable analysis were herd size greater than 2 animals (OR = 2.13, 95% CI 1.61–2.84); breeding method by keeping or hiring bull from neighbouring farm (OR = 3.12, 95% CI 2.40–4.06); extensive grazing on pasture versus intensive zero grazing farming system (OR = 4.44, 95% CI 3.33–5.92); keeping cats against no cat in the farm (OR = 2.41, 95% CI 1.68–3.47); livestock farmers with training on livestock husbandry (OR = 1.95, 95% CI 1.5–2.53); well or river water sources (OR = 1.66, 95% CI 1.28–2.14).

In the final model, we included eleven fixed effects (that is, breed, animal age, livestock training, breeding method, feeding system, distance between farms, farm cat, region, temperature, precipitation and the interaction between temperature and precipitation) and incorporated the dependency among observations by using District, α, as a random effect.

$$Yij \sim \text{Bin}(1, pij)$$

$$E(Yij) =\sim (pij)$$

$$\begin{aligned}
\text{logit}(pij) = &\alpha + \beta_1 \text{ x breed}_{ij} + \beta_2 \text{ x animal age}_{ij} + \beta_3 \text{ x livestock training}_{ij} \\
&+ \beta_4 \text{ x breeding method}_{ij} + \beta_5 \text{ x feeding system}_{ij} + \beta_6 \text{ x distance farms}_{ij} \\
&+ \beta_7 \text{ x farm cat}_{ij} + \beta_8 \text{ x region}_{ij} + \beta_9 \text{ x temperature}_{ij} + \beta_{10} \text{ x precipitation}_{ij} \\
&+ \beta_{11} \text{ x temperature X precipitation}_{ij} \, \alpha_i
\end{aligned}$$

$$\alpha_i \sim N(0, \sigma^2_{\alpha})$$

Where, Yij is the jth ELISA result binomially distributed with a conditional probability, pij, in

Table 1. Univariable associations between *Leptospira* serovar Hardjo seropositive results in dairy cattle and a set of variables. Independence test (fisher exact) two-sided p-values (P-value) is provided for each level.

Variables	Positive animal	Total animal	Prevalence (%), 95% CI	OR, 95% CI	p.value
Breed type					
SHZ-X-Ayrshire	43	433	9.93, 7.28–13.14	Ref	
SHZ-X-Jersey	16	144	11.11, 6.49–17.42	1.13, 0.62–2.08	0.75
SHZ-X-Friesian	180	1415	12.72, 11.03–14.57	1.32, 0.93–1.88	0.13
Indigenous	30	79	37.97, 27.28–49.59	5.55, 3.19–9.65	0.001
Animal sex					
female	251	2007	12.51, 11.09–14.03	Ref	
male	18	64	28.13, 17.6–40.76	2.74, 1.56–4.80	0.001
Animal age*					
≤ 5 years	118	1177	10.03, 8.37–11.88	Ref	
> 5 years	151	891	16.95, 14.54–19.58	1.83, 1.41–2.37	0.001
Abortion in last 12 months					
no	227	1881	12.07, 10.63–13.63	Ref	
yes	42	190	22.11, 16.42–28.68	2.07, 1.43–2.99	0.001
Herd size					
≤ 2	73	871	8.38, 6.63–10.42	Ref	
> 2	196	1200	16.33, 14.28–18.55	2.13, 1.61–2.84	0.001
Livestock training					
no	152	1444	10.53, 8.99–12.22	Ref	
yes	117	627	18.66, 15.68–21.93	1.95, 1.5–2.53	0.001
Breeding method					
use AI	136	1508	9.02, 7.62–10.58	Ref	
keep/hire bull	133	563	23.62, 20.17–27.35	3.12, 2.40–4.06	0.001
Feeding system					
intensive	171	1767	9.68, 8.34–11.15	Ref	
extensive	98	304	32.24, 27.01–37.81	4.44, 3.33–5.92	0.001
Water source					
Tap	143	1319	10.84, 9.21–12.65	Ref	
Well	126	752	16.76, 14.15–19.62	1.66, 1.28–2.14	0.001
Distance between farms					
≤ 100m	135	1511	8.93, 7.54–10.49	Ref	
> 100m	134	560	23.93, 20.45–27.68	3.21, 2.47–4.17	0.001
Farmer with cats in the farm*					
yes	224	1882	11.9, 10.47–13.45	Ref	
no	45	183	24.59, 18.54–31.49	2.41, 1.68–3.47	0.001
Gender based farm management					
female	92	835	11.02, 8.97–13.34	Ref	
male	177	1236	14.32, 12.41–16.4	1.35, 1.03–1.77	0.03
Education					
primary or none	144	1516	9.5, 8.07–11.09	Ref	
post primary	125	555	22.52, 19.11–26.23	2.77, 2.13–3.6	0.001
Region					
Mbeya	11	218	5.05, 2.55–8.85	Ref	
Kilimanjaro	26	520	5, 3.29–7.24	0.99, 0.48–2.04	1
Arusha	25	318	7.86, 5.15–11.39	1.61, 0.77–3.34	0.22
Njombe	16	187	8.56, 4.97–13.52	1.76, 0.8–3.89	0.17

(*Continued*)

Table 1. (Continued)

Variables	Positive animal	Total animal	Prevalence (%), 95% CI	OR, 95% CI	p.value
Tanga	99	523	18.93, 15.66–22.55	4.39, 2.31–8.37	0.001
Iringa	92	305	30.16, 25.06–35.65	8.13, 4.23–15.63	0.001

* indicates where variables are not equal to 2071 due to missing data, **OR** = Odd ratio, **CI** = Confidence interval, **AI** = Artificial insemination

district i, and i = 1, 20, and district, α_i, is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2. Model assumptions were not violated as shown in S1 Fig, and the model explained 29.1% of the variation (conditional R^2) of which 5.9% was due to random effect.

The identified risk factors for antibodies to *Leptospira* in cattle from the multivariable model (Fig 3) included: age equal to or over 5 years (OR = 1.41, 95%CI 1.05–1.9); Indigenous breed (OR = 2.78, 95%CI 1.47–5.26) compared to other breeds, farmers with livestock training (OR = 1.62 95%CI 1.15–2.27); hiring a bull for breeding (OR = 1.91, 95% CI 1.34–2.71), farm without cats (OR = 1.87, 95% CI 1.16–3.02), animals grazed extensively (OR = 2.31, 95% CI
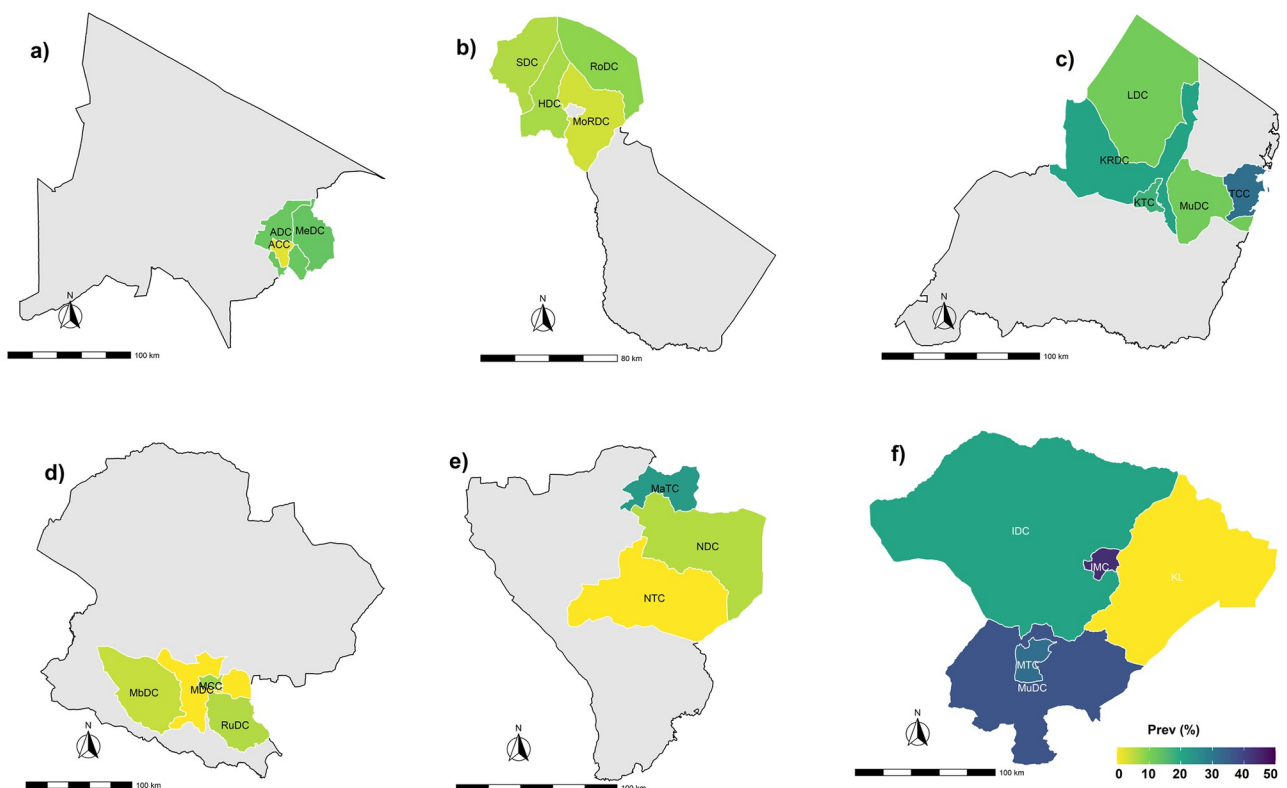

Fig 2. Geographic mapping of leptospirosis distributions and hotspots in 24 districts of study across six regions from two economically important dairy zones of Tanzania. The northern zone (**a**, **b**, and **c**) and southern zone (**d**, **e**, and **f**). **a)** Arusha region consisting of Arusha City Council (ACC), Meru District Council (MeDC), Arusha District Council (ADC), **b)** Kilimanjaro region consisting of Rombo District Council (RoDC), Moshi District Council (MoRDC), Hai District Council (HDC), Siha District Council (SDC), **c)** Tanga region consisting of Tanga City Council (TCC), Muheza District (MuDC), Korogwe District (KRDC), Korogwe Town Council (KTC), Lushoto District (LDC), **d)** Mbeya region consisting of Mbeya District Council (MDC), Mbeya City Council (MCC), Mbozi District Council (MbDC), **e)** Njombe region consisting of Njombe District Council (NDC), Makambako Town Council (MaTC), Rungwe District Council (RuDC), Njombe Town Council (NTC) and **f)** Iringa region consisting of Iringa District Council (IDC), Iringa Municipal Council (IMC), Mafinga Town Council (MTC), Mufindi District Council (MuDC). Map source: data shape file for Tanzania map at all levels downloaded from https://gadm.org/.

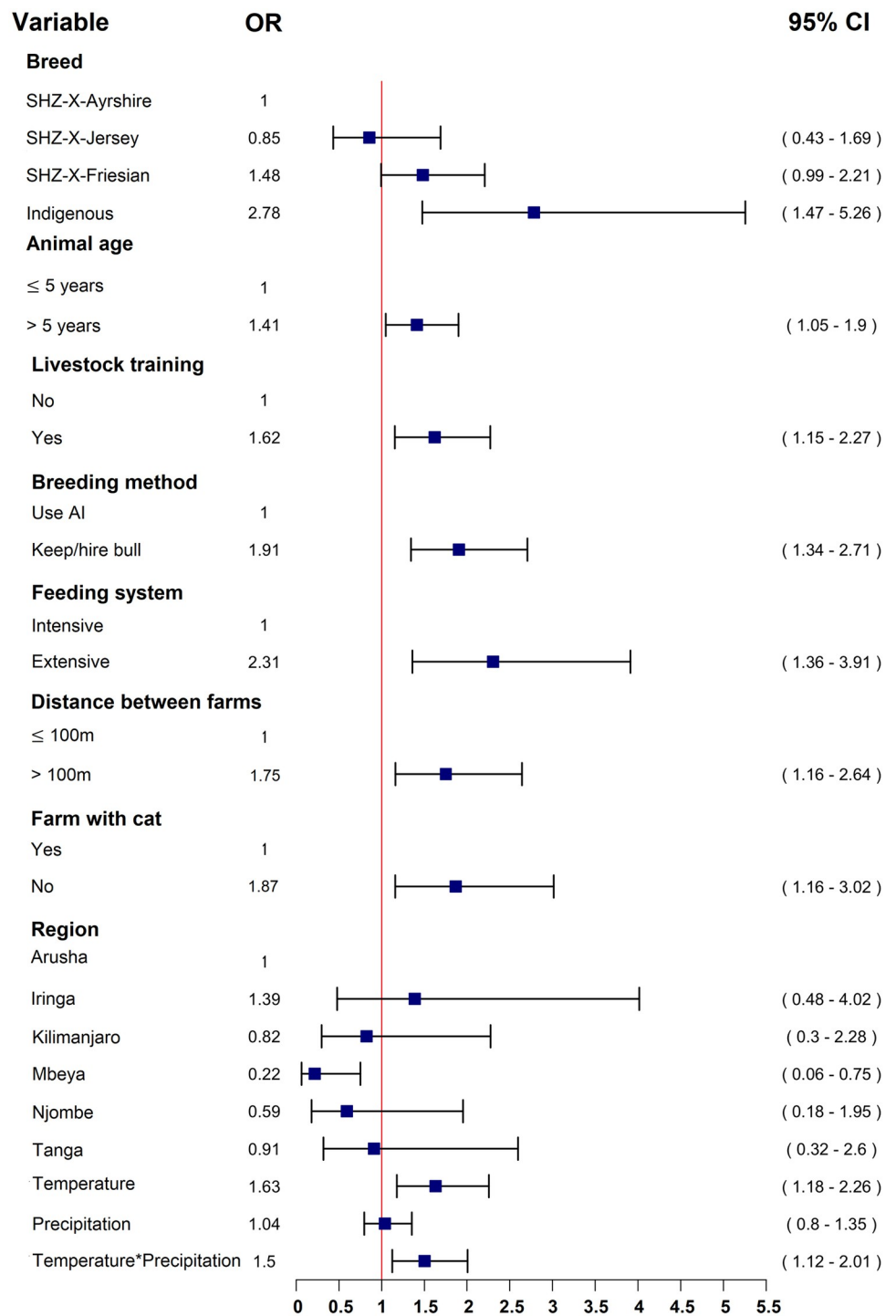| Variable	OR		95% CI
Breed			
SHZ-X-Ayrshire	1		
SHZ-X-Jersey	0.85		(0.43 - 1.69)
SHZ-X-Friesian	1.48		(0.99 - 2.21)
Indigenous	2.78		(1.47 - 5.26)
Animal age			
≤ 5 years	1		
> 5 years	1.41		(1.05 - 1.9)
Livestock training			
No	1		
Yes	1.62		(1.15 - 2.27)
Breeding method			
Use AI	1		
Keep/hire bull	1.91		(1.34 - 2.71)
Feeding system			
Intensive	1		
Extensive	2.31		(1.36 - 3.91)
Distance between farms			
≤ 100m	1		
> 100m	1.75		(1.16 - 2.64)
Farm with cat			
Yes	1		
No	1.87		(1.16 - 3.02)
Region			
Arusha	1		
Iringa	1.39		(0.48 - 4.02)
Kilimanjaro	0.82		(0.3 - 2.28)
Mbeya	0.22		(0.06 - 0.75)
Njombe	0.59		(0.18 - 1.95)
Tanga	0.91		(0.32 - 2.6)
Temperature	1.63		(1.18 - 2.26)
Precipitation	1.04		(0.8 - 1.35)
Temperature*Precipitation	1.5		(1.12 - 2.01)

Fig 3. A forest plot summarizing the final multivariable model of significant predictive variables for leptospirosis association to seropositive occurrence in smallholder Tanzanian dairy cattle.

https://doi.org/10.1371/journal.pntd.0011199.g003

1.36–3.91) and more than 100 meters distance between the farms (OR = 1.75, 95%CI 1.16–2.64). Increase in temperature (OR = 1.63, 95% CI 1.18–2.26), and the interaction between increased temperature and precipitation (OR = 1.50, 95%CI 1.12–2.01) were also found to be significant risk factors (Figs 3 and 4).
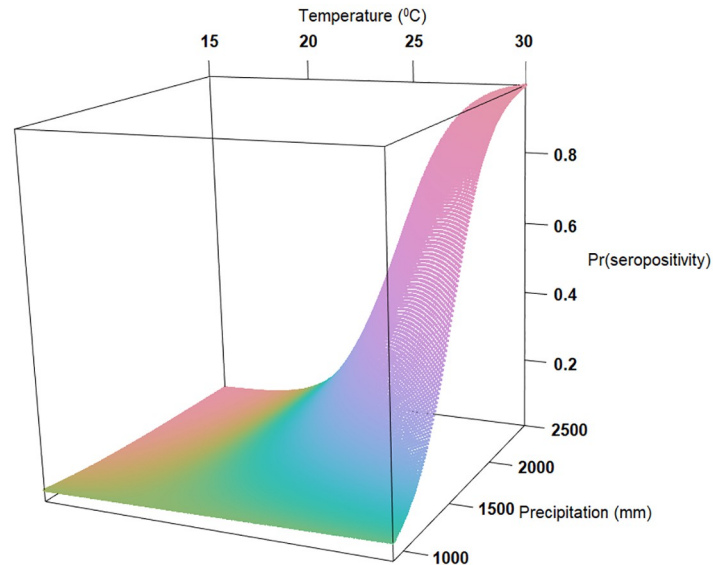
Fig 4. Three-dimensional graph shows the predicted probability of *Leptospira* serovar Hardjo seropositivity, Pr (seropositive), as a result of the interaction of increased temperature (˚C) and precipitation (mm), and accounting for all other fixed effects in final generalised linear mixed effects model.

https://doi.org/10.1371/journal.pntd.0011199.g004

Discussion

This study estimated the seroprevalence of antibodies to *Leptospira* serovar Hardjo and quantified risk factors for exposure in dairy cattle in Tanzania. Given the importance of dairy farming in Tanzania, this study provides important insights and highlights the need for action given the high seroprevalence and identified hotspots of this globally neglected zoonosis.

Here we report the seroprevalence of *Leptospira* serovar Hardjo across the major dairy keeping regions of the northern and southern highlands of Tanzania. To our best understanding and knowledge, this study is the first to describe *Leptospira* seroprevalence in dairy cattle in the Southern Highlands with no previous studies in Iringa or Njombe regions and a previous study in Mbeya reporting one seropositive case [14].

There was a variation in seroprevalence between the regions. For instance, Iringa and Tanga recorded higher seroprevalence than the other regions with (30.2%, 95% CI 25.1–35.7) and (18.9%, 95% CI 15.7–22.6), respectively, and there was significant more risk for cattle raised in Iringa region (OR = 1.39, 95% CI 0.48–4.02) than the other regions included in the study. This is in line with other previous seroprevalence estimates in cattle of 17.59% in Katavi [19] and 15% in Tanga [17], The study observed higher proportions of seropositive cattle from the southern zone 19.8% than the northern zone 11.4%.

The highest leptospirosis seroprevalences globally seem to be in areas characterized by a relatively warm, temperate environment and high precipitation which are essential factors for viable leptospiral maintenance and dissemination [31,32]. Our model (Fig 3) suggested the probability of seropositivity in smallholder dairy cattle increased with higher temperature (OR = 1.63, 95% CI 1.18–2.26). Interestingly, the probability of seropositivity increased significantly (OR = 1.5, 95% CI 1.12–2.01) when both temperature and precipitation increased (Figs 3 and 4). As shown elsewhere [33], leptospirosis outbreaks in our study sites are likely to occur more frequently during the warm rainy season.

Cattle grazed under extensive farming were significantly more likely to be seropositive than cattle from farms practicing intensive grazing (OR = 2.31, 95% CI 1.36–3.91). Similarly, cattle

on farms with a distance between farms of more than 100 meters were at higher risk
(OR = 1.75,95% CI 1.16–2.64) of being seropositive than farms with below 100 meters distance. Farm to farm distance was set to 100m, since the average smallholder farm in Tanzania
is 1.2 hectares, this means that intensively managed animals are unlikely to have direct contact
with each other or share resources [34]. It was observed during the study that farms with
increased distance between farms had greater access to pasture and direct contact between animals of neighbouring farms. In addition to the farm management practices, low biosafety and
biosecurity potentially put dairy cattle at higher risk of leptospirosis and spread in the herd.
These findings complement past studies which concluded that extensive farming practices and
co-grazing encourage pathogen transmission to susceptible animals [35] through contact with
infected animals and access to contaminated pastures and water [17].

Meanwhile, varying sources have reported different susceptibility of cattle to leptospirosis
infection based on the age class. Older animals are more likely to be seropositive than younger
animals [36], In this study dairy cattle with age above 5 years were more likely to be seropositive (OR = 1.41, 95% CI 1.05–1.9) than younger animals aged 5 years and below as previously
described [37]. It should be noted that none of the 1370 study farms had history of vaccination,
treatment, or any control measure against leptospirosis. This suggests that the high seropositivity to *Leptospira* serovar Hardjo in older cattle may be due to the increased possibility of exposure to *Leptospira* in the environment, and also carrier animals in the same herd [11].

Livestock training was an important factor for seropositivity in dairy cattle. Cattle belonging to farmers who received livestock training were at higher risk of being seropositive
(OR = 1.62, 95% CI 1.15–2.27) than cattle belonging to farmers who did not have training on
livestock keeping. This was contrary to expectations that animals belonging to farmers who
received training on dairy keeping would be at lower risk to contract leptospirosis as the farmers abide by farm management and precautionary measures to prevent disease spread. The
higher risk may be attributed to the practice of farmers hiring untrained personnel to take care
of the animals as it was observed during the study.

Livestock production in Tanzania remains a challenge particularly for smallholder dairy
farmers. In this study, several smallholder dairy farmers relied on breeding with a bull, and a
few of them used artificial insemination (AI) which was not easily accessible because of the
limited expertise for this service. Cattle on farms with kept or hired bull for breeding were
more likely to be seropositive than cattle on farms using AI methods for breeding purposes
(OR = 1.69; 95% CI 1.15–2.48). Hiring a bull for breeding in Tanzanian dairy cattle was shown
to be an important factor for disease spread in animals within the herd, and between neighbour farms through sexual contact [38]. It has been reported by previous authors [36], that hiring a bull or close contact between animals for calf raising is the most remarkable determinant
for leptospirosis infection in smallholder dairy farms.

This study found cattle breed was significantly associated with seropositivity with indigenous cattle being significantly more likely to be leptospirosis seropositive (OR = 2.78, 2.48
(95% CI 1.47–5.26) than SHZ-X-Friesian (OR = 1.48, 95% CI 0.99–2.21) or other crossbreeds.
This is contrary to findings in other regions where crossbred cattle have been reported to
have higher seropositivity [39]. Further work is required to understand the increased seroprevalence in indigenous cattle in this setting and if this also relates to increased disease
susceptibility.

This study found that farms that do not keep cats in the farm were significantly more likely
to have seropositive cattle (OR = 1.87, 95% CI 1.16–3.02). Epidemiologically, rodents are
mainly known for carrying different pathogenic *Leptospira* and contaminate pasture [38], consequently livestock may acquire leptospirosis infection during grazing [40]. Keeping of cats in
the farm was likely to reduce the rodent numbers particularly in cow sheds, in the reserved

pastures, or hay barns which could be a protective measure to reduce exposure of cattle to *Leptospira* pathogens.

The findings in this study underpin the importance of leptospirosis in dairy farms. The presence of *Leptospira spp*. in dairy farms has previously been attributed to environmental contamination from the reservoirs of the pathogen and dairy animals that share grazing pastures and the environment [41]. Infected animals can contaminate the environment with leptospires by excretion in urine which can remain infectious in the environment for a few weeks to a month [42]. Contamination of the environment is linked to spread via water sources or animal feeds that can be accessed by other animal species [43]. These may also become sources of infection to animal caretakers or slaughterhouse workers [44]. It has already been highlighted that leptospirosis infection in humans is largely dictated by its prevalence in livestock [45].

Conclusion

This study provides an insight into the epidemiological status and exposure in smallholder dairy cattle raised across the country to *Leptospira* serovar Hardjo. In addition, all dairy cattle in Tanzanian smallholder farms were not vaccinated against leptospirosis. The findings highlight that the disease is prevalent in smallholder dairy cattle population and there were high levels of leptospirosis exposure in specific regions. The disease seropositivity of the studied dairy cattle was significantly associated with individual animal factors such as age and breed, as well as with management practices such as knowledge on animal husbandry, keeping cat for rodent control, breeding practices, and distance between the farms. Precipitation and temperature were also significant environmental risk factors in this cattle population.

Recommendation

The limitation of this study is the focus on only *Leptospira* serovar Hardjo exposure in cattle by ELISA. We recommend further study to identify additional serovars that might be missed from the test and that may be circulating in Tanzania smallholder dairy cattle. It is important for the future studies to consider additional serotyping methods such as microscopic agglutination test [19] or molecular typing [46] to characterize more serovars. For example, due to the fact that these similar regions have records of intensive pig breeding as well as improved dairy cattle, the presence of pigs in or near cattle bomas or farms may increase chances of contact between pigs and dairy cattle and thus spread of *Leptospira* serovars to cattle such as Pomona, Australis, and Tarassovi serovar which are principally maintained by pigs [47].

Moreover, more studies should be carried out with a special focus on human leptospirosis especially in smallholder dairy farmers. The high prevalence of leptospirosis in cattle may play an important role in disease transmission to humans, particularly to livestock keepers and slaughterhouse workers [18]. Generally, individual and community education regarding the risks of leptospirosis disease and prevention measures is recommended to control and prevent the spread of this zoonotic disease.

Supporting information

S1 Table. Model selection results for the generalised linear mixed effect model for Leptospirosis serovar Hardjo in smallholder dairy cattle. The most strongly supported model is number 7. For each model, formula, Akaike information criterion (AIC), and Loglikelihood ratio test p-value (LRT p-value) are provided.
(DOCX)

S1 Fig. Simulation plot of predictable variable to validate best fit of model for leptospirosis occurrence predictions in smallholder dairy cattle in Tanzania.
(TIF)

Acknowledgments

Author Contributions

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Sero-molecular epidemiological analysis of leptospirosis in smallholder dairy cattle in selected regions of Tanzania

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Introduction

The smallholder dairy (SHD) industry is one of fastest-growing industries, accounting for 30% of the entire livestock sector in Tanzania. It is a promising sector for household income generation, creating employment, and improving livelihood especially in rural settings. It is well known *Leptospira* serovar Hardjo in cattle cause economic loses (hard abortion and sharp milk production) from many countries reported before however in Tanzania, the current information best on *Leptospira* serovar Hardjo in cattle are scarce. This study explored the current epidemiological status and estimates prevalence of *Leptospira* srovar Hardjo and identification of additional potential serogroups of *Leptospira* circulating among the Tanzanian smallholder dairy cattle.

Methods

A cross sectional study was carried out in Tanzania dairy cattle. The farmers list were available from the International Livestock Research Institute cattle registry as a subset of the African Dairy Genetics Gains program implemented in six regions of Tanzania (Arusha, Kilimanjaro, Tanga, Mbeya, Iringa and Njombe). The samples collected were tested by three complementary tests (Sandwich ELISA assay, microscopic agglutination test and real-time polymerase chain reaction). Seroprevalence was estimated at different administrative levels and mapped to visualize potential leptospirosis hotspots.

Results

An overall prevalence of 13% (269/2071), 13.1% (202/1494) and 13.7% (286/2086) for *Leptospira* serovar Hardjo by ELISA test, RT-PCR for pathogenic *Leptospira* spp. and microscopic agglutination test (MAT) against five serogroups (Sokoine, Lora, Hebdomadis, respectively.
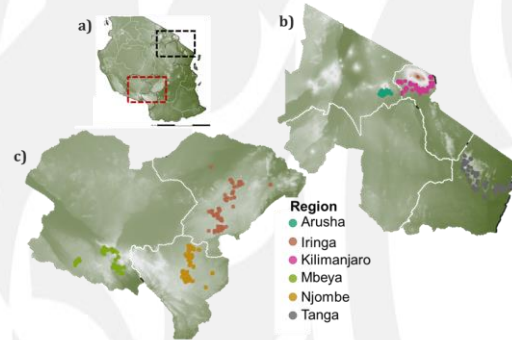

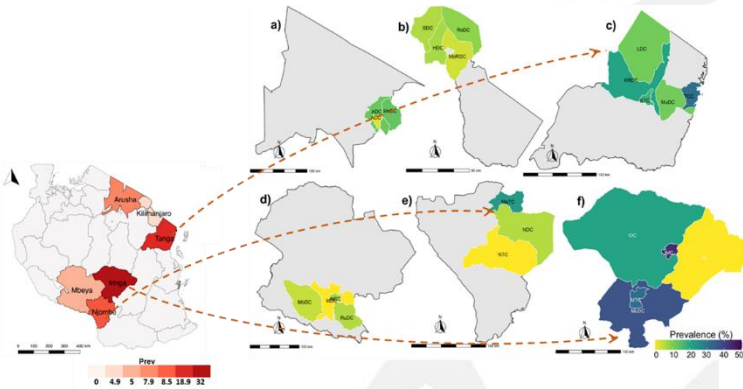**Fig 1. Geographic location of farms, regions, and dairy zones in Tanzania.**

Fig 2. Geographic mapping of leptospirosis distributions and hotspots in 23 districts across six regions from two economically important dairy zones of Tanzania. The northern zone (A, B and C) and southern zone (D, E and F). A) Arusha region of Arusha City Council (ACC), Meru District Council (MeDC), Arusha District Council (ADC), B) Kilimanjaro region of Rombo District Council (RoDC), Moshi Rural District Council (MoRDC), Hai District Council (HDC), Siha District Council (SDC), C) Tanga region of Tanga City Council (TCC), Muheza District (MuDC), Korogwe Rural District (KRDC), Korogwe Town Council (KTC), Lushoto District (LDC), D) Mbeya region of Mbeya District Council (MDC), Mbeya City Council (MCC), Mbozi District Council (MbDC), E) Njombe region of Njombe District Council (NDC), Makambako Town Council (MaTC), Rungwe District Council (RuDC), Njombe Town Council (NTC) and F) Iringa region of Iringa District Council (IDC), Iringa Municipal Council (IMC), Mafinga Town Council (MTC), Mufindi District Council (MuDC), 2020.

Conclusion

In all six regions (Arusha, Kilimanjaro, Tanga, Iringa, Mbeya and Njombe), reveal the *Leptospira* exposure in dairy cattle is high and highlight the need of understanding the disease transmission dynamic between animals and human exposures particularly dairy farmers who works closely to cattle. This findings provide an opportunity for future study in a given that data were limited to *Leptospira* serovar Hardjo and a narrowed panel of only five serogroups from twenty-six known serogroups. A broad serogroups panel to be considered for more identification of common serogroups circulating in cattle foe vaccine target in Tanzania cattle population.

Acknowledgment

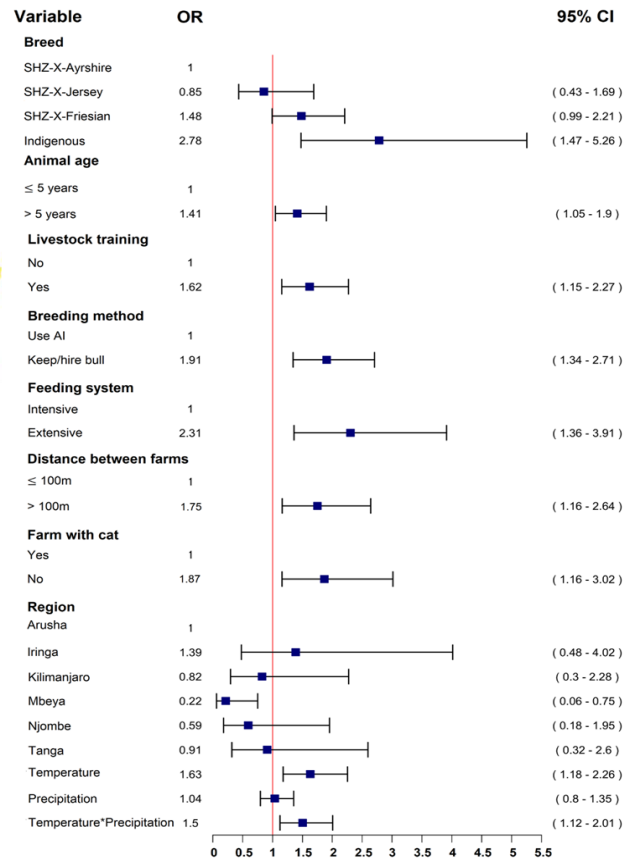| Variable	OR		95% CI
Breed			
SHZ-X-Ayrshire	1		
SHZ-X-Jersey	0.85		(0.43 - 1.69)
SHZ-X-Friesian	1.48		(0.99 - 2.21)
Indigenous	2.78		(1.47 - 5.26)
Animal age			
≤ 5 years	1		
> 5 years	1.41		(1.05 - 1.9)
Livestock training			
No	1		
Yes	1.62		(1.15 - 2.27)
Breeding method			
Use AI	1		
Keep/hire bull	1.91		(1.34 - 2.71)
Feeding system			
Intensive	1		
Extensive	2.31		(1.36 - 3.91)
Distance between farms			
≤ 100m	1		
> 100m	1.75		(1.16 - 2.64)
Farm with cat			
Yes	1		
No	1.87		(1.16 - 3.02)
Region			
Arusha	1		
Iringa	1.39		(0.48 - 4.02)
Kilimanjaro	0.82		(0.3 - 2.28)
Mbeya	0.22		(0.06 - 0.75)
Njombe	0.59		(0.18 - 1.95)
Tanga	0.91		(0.32 - 2.6)
Temperature	1.63		(1.18 - 2.26)
Precipitation	1.04		(0.8 - 1.35)
Temperature*Precipitation	1.5		(1.12 - 2.01)

Fig 3. A glmmTMB forest plot summarizing significant predictive variables for leptospirosis and association to seropositivity in smallholder Tanzanian dairy cattle