PREVALENCE AND ECONOMIC EFFECTS OF NON TYPHOIDAL *Salmonella* INFECTION IN WILD AND DOMESTIC ANIMALS AROUND RUAHA NATIONAL PARK ECOSYSTEM, TANZANIA

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EXTENDED ABSTRACT

Non-typhoidal *Salmonella* infection is one of the zoonotic conditions caused by Gram negative bacteria of the genus *Salmonella*, which belong to the family *Enterobacteriaceae*. Non-typhoidal *Salmonella* infection is an important foodborne infection particularly in immunocompromised humans. This study assessed the prevalence and economic impact of non-typhoidal *Salmonella* infections in wild and domestic animals around the Ruaha National Park Ecosystem and the study was conducted in 2021. A laboratory analysis of 215 fecal samples from wild animals (108 samples) and domestic animals (107 samples) was carried out to determine the prevalence of non-typhoidal *Salmonella* infection after the samples were enriched in Selenite F broth and incubated for 24 hours. The samples were primarily cultured on the Salmonella Shigella agar (SSA) and subsequently on MacConkey and blood agar to observe any swarming. Gram staining was conducted to check whether the suspected colonies were Gram negative since *Salmonellae* belong to this group. Confirmatory biochemical tests were conducted using the Triple Sugar Iron (TSI) test, IMViC tests, oxidase and catalase tests and molecular confirmation was done by extracting the DNA followed by a conventional multiplex PCR. Laboratory analysis revealed that non-typhoidal *Salmonella* infection was absent in the wild and domestic animals. A structured questionnaire was administered to assess the awareness and economic impact of non-typhoidal *Salmonella* infection. Analysis of Variance (ANOVA) test was done to assess the awareness, estimated costs of resources lost as well as the costs used for treatment of animals with non-typhoidal *Salmonella* infections at P-value 0.05. Findings from this study revealed that for the past five years until year 2020, 20% of the livestock keepers were aware of non-typhoidal *Salmonella* infection. The study showed that for the past five years until year 2020 the infection had negative economic impact since 102,140 Tsh. was estimated to be spent per year in controlling non-typhoidal *Salmonella* infection while 680,100 Tsh. was the estimated amount of lost resources because of non-typhoidal *Salmonell*a infection. Therefore, from the findings, we recommend that the responsible government sectors should invest in more research on non-typhoidal *Salmonella* infection on other national park ecosystems as well as other zoonoses but also add effort in increasing awareness of zoonoses to the communities. This will help livestock keepers improve the animal rearing methods hence reducing the incidences of zoonotic diseases particularly non-typhoidal *Salmonella* infection.

DECLARATION

I, Zamu Mdindikasi, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
BA	Blood agar
DCA	Deoxycholate Citrate agar
Df	Degree of freedom
DF	Dengue fever
ID	Identity
MDR	Multiple drug resistance
NTS	Non typhoidal *Salmonellae*
PCR	Polymerase chain reaction
SPSS	Statistical Package for Social Sciences
SSA	Salmonella Shigella agar
TANAPA	Tanzania National Parks
TSI	Triple Sugar Iron
Tsh.	Tanzanian Shillings
USA	United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Non-typhoidal *Salmonella* infection is one of the zoonotic conditions caused by bacteria of the genus *Salmonella*, which are transmitted from animals to humans hence posing public health threats around the globe (Graham, 2002). In 2010, the World Health Organization estimated that about 600 million diseases cases were caused by contaminated food, with about 350 million of these being caused by pathogenic bacteria (Chlebicz and Śliżewska, 2018). *Salmonella* infection is generally said to be of high risk in areas with large concentrations of humans and animals, untreated manure and sewage contaminating the surroundings (D'Aoust and Maurer, 2007).

The genus *Salmonella* belongs to the family *Enterobacteriaceae*, consisting of non-spore-forming, facultative anaerobic, Gram-negative bacilli, which move by peritrichous flagella (Yoshikawa *et al.,* 1980). *Salmonella* bacteria may dwell in domestic animals, such as poultry, cattle, and swine and are also found in wild animals, pets, fish, and rodents. Infected animals are often asymptomatic carriers of the pathogens and excrete the pathogens in feces, thus releasing the bacteria to the environment (Chlebicz and Śliżewska, 2018). *Salmonella* can survive for some time without the need of a host and inhabit polluted water with contamination from the excrement of carrier animals being particularly an important source (Morpeth *et al*., 2009). Since *Salmonella* infections can affect wild and domestic animals as well as humans, they are important in public health.

Infection to humans is usually through consumption of meat from wild and domestic animals, or eggs and milk which have been contaminated with the bacteria from domestic animals. Non-typhoidal *Salmonella* infection in humans presents with; gastroenteritis, enteric fever, bacteremia, diarrhea and stomach cramps, usually beginning from six hours to six days after infection (Yoshikawa *et al*., 1980).

In Tanzania, previous studies have shown that invasive non-typhoidal *Salmonella* infection is an important cause of hospitalized febrile diseases among children**.** Invasive non-Typhi serotypes (NTS) salmonellosis disease is associated with a high risk of death (Mtove *et al.,* 2010). Lubote and colleagues reported that the prevalence of *Salmonella* species in some parts of Tanzania ranged from 0.17% to 28% (Lubote *et al.,* 2014)**.** The objective of this study, therefore, was to assess the prevalence of non-typhoidal *Salmonella* infection in wild and domestic animals and its economic impact in communities around the Ruaha National Park Ecosystem. The study findings will help the responsible government sectors increase focus on zoonotic disease control as well as increase of awareness on the existence of zoonoses, particularly non-typhoidal *Salmonella* infection in the communities around Ruaha National Park Ecosystem and elsewhere.

1.1 *Salmonella* species

Salmonella spp*.* are facultative anaerobic gram-negative rod-shaped bacteria which belong to the family *Enterobacteriaceae* (D'Aoust and Maurer, 2007). They are classified into more than 2500 serovars based on the Kauffmann–White scheme (Popoff *et al.,* 2003) and some have been defined as major pathogens to humans and animals including *Salmonella typhimurium*, *S. enteritidis* and *S. typhi*. The *Salmonellae* are divided taxonomically into two species: *Salmonella enterica* and *Salmonella bongori* (subspecies V). *Salmonella enterica* comprises six subspecies, which are *S. enterica* ssp. *enterica* (I), *S. enterica* ssp. *salamae* (II), *S. enterica* ssp. *arizonae* (IIIa), *S. enterica* ssp. *diarizonae* (IIIb), *S. enterica* ssp. *houtenae* (IV) and *S. enterica* ssp. *indica* (VI).

Salmonella enterica serovar *typhimurium* phage types DT104 and U302 are considered zoonotic and life-threatening pathogens. These strains are commonly associated with multiple-drug-resistant (MDR) potential carried by genetic elements in their chromosomes (Amavisit, 2005). As it was reported by Li *et al*. (2012), the antibiotic resistance in *Salmonella* spp, has been reported since early 1960's.

The widespread occurrence of *Salmonellae* in the environment and their prevalence in the food chain has made the persistence of *Salmonella* spp among the leading zoonotic foodborne illnesses for many years (Li *et al.*, 2012).

1.2 Non-typhoidal *Salmonella* species

Non-typhoidal *Salmonellae* (NTS) are *Salmonella* serovars other than *Salmonella enterica* serovar *Typhi* (*S. Typhi*) and the various pathovars of *S. Paratyphi* which are commonly referred to as typhoidal *Salmonella* serovars and commonly restricted to human hosts. These non-typhoidal *Salmonella* (NTS) serovars have the potential to interact with human and nonhuman hosts (Haselbeck *et al*., 2017).

Non-typhoidal *Salmonella* spp*.* are important foodborne pathogens that cause gastroenteritis, bacteremia, and subsequent focal infection. These bacteria are a big problem in a wide variety of immunocompromised individuals, including patients with HIV or diabetes, and those receiving corticosteroid therapy or treatment with other immunotherapy agents (Acheson and Hohmann, 2001).

It is estimated that 93.8 million cases (5th to 95th percentile, 61.8 to 131.6 million) of gastroenteritis due to non-typhoidal *Salmonella* species occur globally each year, with 155,000 deaths (5th to 95th percentile, 39 000–303 000 deaths). Of these, it is estimated that 80.3 million cases are foodborne. Non-typhoidal *Salmonella* infection represents a considerable health burden globally (Majowicz *et al*., 2010).

1.3 Reservoir of *Salmonellae*

Wild animals are the principal source of infection of many important zoonotic diseases including bovine tuberculosis, salmonellosis, avian influenza, swine flu and rabies (Simpson, 2002). However, among reservoirs of *Salmonellae* and common source of *Salmonella* infections are poultry (Nurmi and Rantala, 1973). These authors reported a severe outbreak of *Salmonella infantis* infection among Finnish broiler flocks and 277 people were diagnosed to have *Salmonella* infection caused by the same serotype in 1971. An outbreak of *Salmonella typhimurium* infection in cats and humans in 1999 was associated with wild birds. Salmonellosis was transmitted from cats to humans, but there were only a few such cases (Tauni and Österlund, 2000).

Salmonella enterica, var Typhimurium, is commonly found in the intestine of wild birds. These organisms are maintained within bird populations by several mechanisms. The simplest of these mechanisms occurs in raptors because birds that eat other animals risk eating *Salmonella*-infected prey. Wild and captive raptors may be transient or permanent *Salmonella* carriers or even suffer from clinical salmonellosis as a result of eating infected prey (Tizard, 2004).

1.4 *Salmonella* infection

When ingested organisms (*Salmonellae*) bypass gastric defenses, they multiply in the intestinal lumen and subsequently penetrate the intestinal mucosa, followed by multiplication within macrophages of the reticuloendothelial system. Thereafter, they may be disseminated via the systemic circulation. Invasive strains of non-typhoidal *Salmonellae*, like the distinct genotype of *Salmonella enterica* var Typhimurium and ST313, are reported as prominent causes of bloodstream infection in African adults and children (Feasey *et al.*, 2012; Ao *et al*., 2015). The pathological and clinical manifestations of salmonellosis include enteric fever, gastroenteritis, bacteremia with or without metastatic disease and symptomatic carriage.

However, *Salmonella* infection by some serotypes include serious complications of bacteremia such as infections of the aorta, endocardium, bone and meninges. *Salmonella* infection can be severe in victims with other diseases including AIDS, leukemia, lymphoma, immunodeficiency, inflammatory bowel disease, schistosomiasis and macrophage dysfunction (Goldberg and Rubin, 1988).

Chloramphenicol, ampicilin, amoxillin and trimethoprimsufamethoxazole have shown efficacy in treatment of salmonellosis (Goldberg and Rubin, 1988). Non typhoidal *Salmonella* infections most commonly occur in children below the age of 2 to 3 years and are an important cause of neonatal sepsis in Africa (Gordon, 2011). However, they are relatively uncommon in the first 3 to 4 months of life.

Non-typhoidal salmonellosis is the most important bacterial foodborne infection in humans. *Salmonella* resides in a variety of hosts, including animals living in the wild. There are different ways in which wildlife are involved in human salmonellosis transmission including contact with domestic animals as transmission or accumulation vectors; by contact with humans through meat of wild animals and by contamination of food or food producing units (Hilbert *et al*., 2012).

1.5 Indicator organism for *Salmonellae*

One of the groups of organisms which serve as indicator of *Salmonellae* in a sample is *Escherichia coli*. *Salmonellae* and *E. coli* are said to exist in the same environments since the host environment provides the microorganisms with a warm constant temperature, as well as high concentrations of free amino acids and sugars, which are conducive to bacterial growth (Winfield and Groisman, 2003). Once excreted from an animal host, *Salmonellae* and *E. coli* have to survive under limited nutrient availability, osmotic stress, large variations in temperature and pH. It has been proposed that bacteria can survive such stressful conditions by entering a viable but non-culturable (VBNC) state. The VBNC hypothesis describes an apparent dormant state in which bacterial cells are metabolically active but cannot be cultured by known laboratory methods. It is suggested that bacteria enter this state in response to harsh environmental conditions, such as a temperature change, high salinity, or nutrient deprivation (Winfield and Groisman, 2003). When cultured in Salmonella-Shigella agar, *E. coli* present differently from *Salmonellae*, the former appearing as slight pink smooth colonies while the later appears as black colonies (Islam *et al*., 2014).

1.6 *Salmonella* detection and bacterial culture

Salmonella infection is commonly detected by examining a fecal sample, but if the infection is in the bloodstream, then testing a sample of blood for the bacteria is needed (Baron *et al.,* 1996). The most commonly used culture media for isolating *Salmonella* from specimens and corresponding colony characteristics are: (i) Salmonella Shigella agar which show the *Salmonella* as colonies with black centers (ii) Blood Agar on which *S. typhi* and *S. paratyphi* usually produce non-hemolytic smooth white colonies (iii) MacConkey Agar in which the non-lactose fermenting *Salmonellae* show smooth pale colonies (iv) Deoxycholate Citrate Agar (DCA) which show the *Salmonella* as pale colonies (v) Diagnostics Brilliant Green Agar is the selective enrichment medium for *Salmonella* spp. other than *S. typhi* and *S. paratyphi* from clinical and non-clinical specimens. In this medium *Salmonella* spp. and other non-lactose-fermenters appear as red to pink-white colonies surrounded by brilliant red zones in the medium while lactose-fermenting or sucrose-fermenting organisms appear as yellow to yellow-green colonies surrounded by yellow-green zones in the medium (Chan *et al*., 2003). For rapid detection of *Salmonella* spp, culture procedures can be complemented with PCR techniques to amplify the genus-specific invE/invA genes (Schrank, 2001).

1.7 Economic impact of *Salmonella* Infection

Salmonella infection impacts negatively on the economy of an area where it occurs. A study was conducted to analyse the economic effects of introducing alternative *Salmonella* control strategies in Sweden and the net effects (benefits minus costs) were negative in all scenarios (€ −5 to −105 million), implying that it would not be cost-effective to introduce alternative control strategies in Sweden. This result was mainly due to an expected increase in the incidence of *Salmonella* in humans (6035 to 57108 new cases per year), with expected additional costs of € 5 to 55 million. It was noted that *Salmonella* infection always leads to a negative impact economically, especially because there is crucial need for control in case of any outbreak but also loss of resources and human productivity when deaths occur (Sundström *et al*., 2014).

1.8 Problem Statement and Justification

Zoonotic infections are severely debilitating to humans and animals and sometimes lead to prolonged suffering and deaths. Non-typhoidal salmonellosis as one of the zoonotic infections is of significant importance due to high mortality and morbidity to susceptible victims, resulting to about 2.2 million human deaths worldwide annually (Chlebicz and Śliżewska, 2018).

In Tanzania, invasive non-typhoidal salmonellosis is an important cause of hospitalized febrile diseases among children, with a great risk of death (Mtove *et al.,* 2010). However, the extent to which non-typhoidal *Salmonella* infection is spread among domestic and wild animals and its economic impact to the communities around National Park Ecosystems is not clearly understood.

This study, therefore, assessed the prevalence of non-typhoidal salmonellosis in 2021 and its economic impact for the past five years amongst Idodi division communities as

Idodi division comprises of wards with villages located close to the Ruaha National Park. Data generated from this study are expected to help the policy makers and other stakeholders make informed decisions for the welfare of the surrounding communities and the nation at large.

1.9 Objectives

1.9.1 Main objective

To assess the prevalence and economic impact of non-typhoidal salmonellosis in wild animals and domestic animals at their interface in Ruaha National Park Ecosystem

1.9.2 Specific objectives

i. To assess the prevalence of non-typhoidal *salmonella* infection in wild and domestic animals at the interface in Ruaha National Park Ecosystem.
ii. To assess the awareness and economic impact of non-typhoidal *salmonella* infection to the communities around Ruaha National Park Ecosystem.

1.9.3 List of manuscripts

i. Prevalence of Non-Typhoidal *Salmonella* Infection in Wild and Domestic Animals Around Ruaha National Park Ecosystem, Tanzania
ii. Community Awareness and Economic impact of Non-Typhoidal *Salmonella* Infection in Wild and Domestic Animals around Ruaha National Park Ecosystem, Tanzania

CHAPTER TWO

Manuscript One

2.0 Community Awareness and Economic Impact of Non-Typhoidal *Salmonella* Infection in Wild and Domestic Animals around Ruaha National Park Ecosystem, Tanzania

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Abstract

Non-typhoidal *Salmonella* infection is one of the zoonotic infections caused by Gram -negative bacteria of the genus *Salmonella,* which belongs to the family *Enterobacteriaceae*. Non typhoidal *Salmonella* infection is an important bacterial foodborne infection causing disease in humans, particularly immuno-compromised individuals. This study assesses the community awareness and economic impact of non-typhoidal *Salmonella* infection in wild and domestic animals neighbouring the Ruaha National Park Ecosystem. A structured questionnaire was administered to assess the awareness and economic impact of non-typhoidal *Salmonella* infection in 2021. Analysis of Variance (ANOVA) test was applied to assess the level of awareness, estimated costs of resources lost as well as the costs used for treatment of animals suffering from non-typhoidal *Salmonella* infections at P-value 0.05. Findings from this study revealed that 20% of the livestock keepers were aware of non-typhoidal *Salmonella* infection based on the clinical symptoms manifested by the animals. The study showed that the infection had a negative economic impact since 102,140 Tsh. was the estimated amount spent per year in controlling non-typhoidal *Salmonella* infection in domestic animals and 680,100 Tsh. was the estimated amount of lost resources (dead domestic animals) because of non-typhoidal *Salmonella* infection per year. Therefore, from the findings, we recommend that the government invests more in research on non-typhoidal *Salmonella* infection as well as other zoonoses. It is further recommended to increase awareness to the communities about the zoonoses to help livestock keepers improve the animal rearing methods, hence reducing the incidences of zoonotic diseases particularly non-typhoidal *Salmonella* infection.

Keywords: Community awareness, Economic effects, Non-typhoidal *Salmonellae*, zoonotic infections

2.1 Introduction

Non-typhoidal *Salmonella* infection is one of the zoonotic agents caused by bacteria of the *Salmonella* group. The infection can be transmitted from animals to humans making it a big threat to the health and lives of humans globally [1]. *Salmonella* infection is generally of great risk in areas with large concentrations of humans and animals as well as untreated manure and sewage in the surroundings. The World Health Organization estimated in 2010 that about 350 million cases were caused by bacterial contamination alone [2,3].

Salmonellae belong to the *Enterobacteriaceae* family; they are non-spore-forming, facultative anaerobic, Gram-negative bacilli motile by peritrichous flagella [4]. *Salmonella* bacteria may dwell in livestock, such as poultry, cattle, and swine but are also found in wild animals, pets, fish, and rodents. Animals are often asymptomatic carriers of the pathogens; which they excrete in feces, thus releasing the bacteria to the environment [3].

Salmonella infection can affect animals as well as humans, therefore it is an important infection in wildlife and in neighbouring communities. Infection to humans is usually transmitted by eating game meat or contaminated meat, eggs, and milk from domestic animals [4].

Non-typhoidal *Salmonella* infection may present with diverse clinical syndromes, however, diseases caused by non-typhoidal *Salmonella* infection are: gastroenteritis, enteric fever, bacteremia, diarrhea and stomach cramps which usually begin from six hours to six days after infection. Both focal infection and chronic carrier state have been described [4].

Several reports have shown that invasive non-typhoidal *Salmonella* infection is an important cause of hospitalized febrile diseases among children in Tanzania, with the prevalence of 0.17 to 28% [5,6]. Nevertheless, the community level of awareness and economic impact of non-typhoidal *Salmonella* infection (NTS) are not clearly understood.

This study, therefore assesses, the community awareness and economic impact of non-typhoidal *Salmonella* infection in communities around Ruaha National Park Ecosystem. A set of methods were applied to assess the community awareness of non-typhoidal *Salmonella* infection as well as its impact on their economy. This was achieved by administering a structured questionnaire to respondents from five villages around Ruaha National Park Ecosystem.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in the Ruaha National Park Ecosystem at Idodi division, Iringa region in the Southern Highlands Zone of Tanzania, where there is high interaction between domestic animals from the villages and wild animals from the National Park. Ruaha National Park is the largest national park in Tanzania which covers an area of about 20,226 square kilometers. It is located in the middle of Tanzania about 130 kilometers from Iringa town. The Park is one of the Tanzania birds' paradise with more than 571 species. It is a park with magnificent mammals, reptiles and amphibians [7].

The study was carried out in five villages namely; Idodi, Mapogolo, Tungamalenga, Malinzanga and Mafulutolo. These villages are located in two wards of Idodi division which are Idodi and Mlowa.

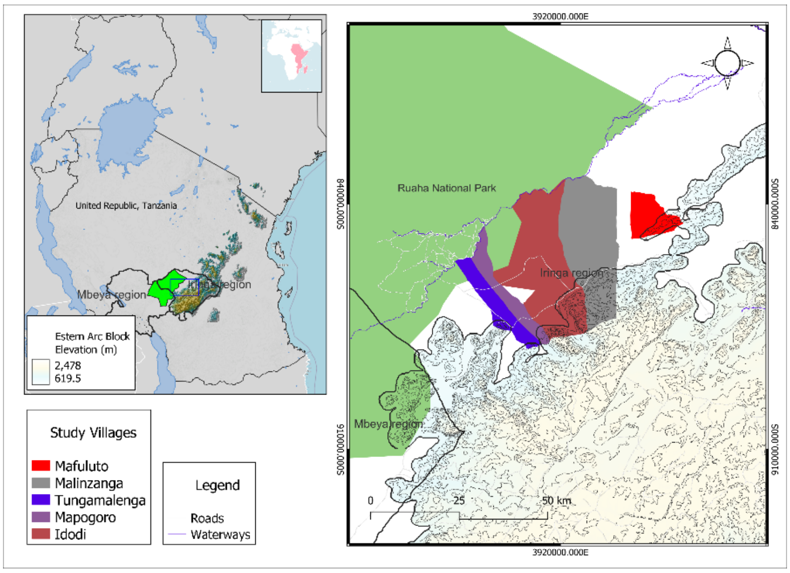

Figure 2.1: A map of Tanzania showing the Ruaha National Park Ecosystem and the study sites

2.2.2 Study design

A cross-sectional study design was used for which five villages from Idodi division were selected purposively. The selection was based on the fact that Idodi division is located in the interface close to Ruaha National Park and the other criteria was the prevalence of salmonellosis and other zoonotic diseases in this division from the previous studies.

2.2.3 Sample size, inclusion and exclusion criteria

The sample size was determined by using Cochran's formula $n= [Z^2P\ (1-P)]/d^2$ [17] where, n =sample size; Z= statistic for level of confidence (1.96); P= prevalence (16.8% as described by [8]; d=precision (5%). The actual sample size was 215 respondents from all five villages. Villages in the interface were included in the study since it is at this location where there is higher interaction between wild and domestic animals. Villages away from the interface were excluded from this study.

2.2.4 Sample collection and sampling strategy

Opportunistic random sampling technique was used to select households for the questionnaire survey.

2.2.5 Questionnaire administration

Knowledge on non-typhoidal *Salmonella* infection and awareness of associated economic losses were assessed by use of structured questionnaire as shown in Appendix 1. The structured questionnaire was given to respondents in their homes and animal keeping sites. One literate representative from each household was chosen by the household members to represent the whole family and the questionnaire was translated to Kiswahili language in order to help the respondents understand the questions and respond accordingly.

A total of 215 respondents from five villages of Mlowa and Idodi wards, located in Idodi division, responded to the questionnaire. Each village provided 43 respondents.

2.2.6 Data analysis

Data from the questionnaire survey were collected, entered and stored into the spread sheet using Microsoft Excel version 12 of 2007 and analysed using a Statistical Package for Social Sciences (SPSS) version 23.0. Descriptive statistics was used to describe the awareness of respondents on any zoonotic diseases and awareness of respondents on non-typhoidal *Salmonella* infection. Analysis of Variance (ANOVA) was done to test the statistical difference in amounts of revenues earned from wild animals or wild animal products as income source to the respondents, estimated costs of resources lost due to death of domestic animals due to non-typhoidal *Salmonella* infections as well as the costs used for treatment of the animals suffering from non-typhoidal *Salmonella* infections at P-value 0.05.

2.3 Results

2.3.1 Profile of the respondents

A total of 215 respondents from the five study villages were interviewed of which 124 (57.7%) of all respondents were married males and females with ages ranging between 30 to 50 years. However, 74.2% of 124 respondents were males and 25.8% of 124 were females. However, 7.0% of all the respondents were at the age above 51 years comprising 12 married males and 3 married females. All interviewed candidates responded that they had at some point raised animals either commercially or for family consumption. All respondents replied that they had never obtained any income from wild animals or any wildlife products. Their income was from agricultural productions or domestic animals or their products (Table 2.1).

Table 2.1: Gender profile of questionnaire respondents

Marital status	Age category	Sex of respondents Male count (Percentage)	Female count (Percentage)	Total count	Total count percentage
Married	19 – 29	16(32.7)	33(67.3)	49	22.8
	30 – 50	92(74.2)	32(25.8)	124	57.7
	51 – Ab0ve	12(80.0)	3(20.0)	15	7.0
Single	19 – 29	20(90.9)	2(9.1)	22	10.2
	30 – 50	5(100)	0(0.00)	5	2.3
	51 – Above	0(0.00)	0(0.00)	0	0.00

2.3.2 Awareness of the livestock keepers (respondents) on zoonotic infections

Respondents with the age ranging from 19 years to 65 years indicated that most of the villagers who keep animals have heard that there are diseases which can be transmitted from animals to humans. Of the 215(100%) respondents interviewed from the five villages, all (100%) have heard of existence of zoonotic diseases, much as they did not mention any disease by name, symptom or modes of transmission of such diseases.

Among the common zoonotic diseases, the majority of respondents (98.1%) seemed to be aware of dengue fever. Rabies, bird flu and anthrax are some of the common zoonotic diseases that were well known among the respondents by 61%, 58% and 53%

respectively. The other common zoonotic diseases were not well known as only a few respondents were aware of these zoonotic diseases. They included swine flu (43.3%), Rift valley fever (22.8%), brucellosis (21.4%), bovine tuberculosis (16.8%), *Salmonella* infection (13.0%) and worms (7.4%). None of the respondents were aware or had ever heard of Q fever (Table 2.2).

Table 2.2: Awareness by respondents of common zoonotic diseases

Zoonotic disease	Awareness on the disease		Total
	Aware	**Unaware**	
Brucellosis	46 (21.4)	169 (78.6)	215 (100)
Bovine TB	36 (16.8)	179 (83.2)	215 (100)
Swine flu	93 (43.3)	122 (56.7)	215 (100)
Bird flu	126 (58.6)	89 (41.4)	215 (100)
Dengue fever	211 (98.1)	4 (1.9)	215 (100)
Q fever	00 (00)	215 (100)	215 (100)
Rabies	133 (61.9)	82 (38.1)	215 (100)
Rift valley fever	49 (22.8)	166 (77.2)	215 (100)
Anthrax	114 (53.0)	101 (47.0)	215 (100)
Salmonella infection	28 (13.0)	187 (87.0)	215 (100)
Others (Worms)	16 (7.4)	199 (92.6)	215 (100)

2.3.3 Opinions of livestock keepers on incidences of zoonotic infections in their area

Majority (79.5%) of the livestock keepers from the five villages of Idodi and Mlowa wards believe that the incidences of zoonotic infections have greatly decreased while 11.6% believed the incidences of zoonotic infections have slightly decreased. Only a small number of livestock keepers believed that the incidences of zoonotic infections has remained unchanged (Table 2.3).

Table 2.3: Views regarding the incidences of zoonotic infections before and after 2020

Diseases incidences	Village ID					Total count	Total count (%)
	Mafuluto	**Malinzanga**	**Idodi**	**Mapogoro**	**Tungamalenga**		
Have decreased substantially	30 (17.5)	34 (19.9)	34 (19.9)	37 (21.6)	36 (21.1)	171	79.5
Have decreased slightly	11 (44.0)	5 (20)	3 (12)	3 (12)	3 (12)	25	11.6
Have remained almost unchanged	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3	1.4
Have slightly increased	0 (0.0)	0 (0.0)	4 (57.1)	1 (14.3)	2(28.6%)	7	3.3
Have increased substantially	2 (22.2)	1 (11.1)	2 (22.2)	2 (22.2)	2 (22.2)	9	4.2
Total	43 (20)	43 (20)	43 (20)	43 (20)	43 (20)	215	100

2.3.4 Response on awareness of non-typhoidal *Salmonella* infection

Livestock keepers from the five villages when asked about their awareness on non-typhoidal *Salmonella* infection specifically, only 13% of respondents were aware of non-typhoidal *Salmonella* infection and they became aware through the symptoms shown by infected animals which were well described by the veterinary doctors. However, a big number of people (87%) responded that they had never heard of non-typhoidal *Salmonella* infection (Table 2.4).

Table 2.4: Awareness of livestock keepers on non-typhoidal *Salmonella* infection

Awareness on non typhoidal *Salmonella* infection	Village ID					Total count	Total count (%)
	Mafuluto	Malinzanga	Idodi	Mapogoro	Tungamalenga		
Yes	9* (32.1)	4* (14.3)	5*(17.9)	6*(21.4)	4*(14.3)	28*	13.0
No	34(18.2)	39(20.9)	38(20.3)	37(19.8)	39(20.9)	187	87
Total	43(20)	43(20)	43(20)	43(20)	43(20)	215	100

*Their animals suffered; they knew about non-typhoidal *Salmonella* infection through symptoms described by the veterinary doctors

2.3.5 Response on the cases of non-typhoidal *Salmonella* infections and their economic impact

Most of the livestock keepers responded that their animals have never suffered from non-typhoidal *Salmonella* infection. In the five villages, the largest number of animals especially chicken and pigs to ever have suffered from non-typhoidal *Salmonella* infection was 110 in 2020 as per the respondents. In five consecutive years, there had been some cases of non-typhoidal *Salmonella* infections in which some animals were treated (Table 2.5)**.** According to the interviewed livestock keepers, they had spent money to treat animals. For example, in 2020, about 274,000 Tsh. was used to buy antibiotics to treat the domestic animals. (Table 2.5).

However, despite treatment, some animals died. At least 18 animals died due to non-typhoidal *Salmonella* infections each year. For example, in 2020, 65 newly born animals died. It was estimated that at least 400,000 Tsh. was spent for treatment each year. In 2020, the estimated loss due to animal mortality due to NTS infection was 1,233,000 Tsh. (Table 2.5).

Table 2.5: Non-typhoidal *Salmonella* infection cases and the costs incurred

Year	No. of animals suffered from non-typhoidal *Salmonella* infection	Total cost of treatment	No. of animals dead/resources lost due to non-typhoidal *Salmonella* infection	Estimated total costs of lost resources
2017	21	10000	18	682000
2018	66	136500	32	400000
2019	73	90200	38	989500
2020	110	274000	65	1233000
2021	8	0	8	96000
Total cost in five years		510700		3400500

2.3.6 Community level of understanding and awareness of zoonoses and non-typhoidal *Salmonella* infection

Majority of the people interviewed had low level of understanding about the zoonotic infections and specifically non-typhoidal *Salmonella* infections. This is indicated by the fact that the calculated P-value (0.191) was greater than the set P-value (0.05) hence the null hypothesis was accepted thus indicating that there is no statistical significant difference in the level of awareness and understanding about the zoonoses and non-typhoidal *Salmonella* infection referring to the total score of the administered questionnaire as shown in the ANOVA table (Table 2.6).

Table 2.6: ANOVA results indicating the total score analysis

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	580.540	4	145.135	1.542	0.191
Within Groups	19760.186	210	94.096		
Total	20340.726	214			

However, in spite of the fact that there was no statistical significant difference in the level of awareness among the respondents from all villages, 44 people (20.5%) had a higher level of awareness about non-typhoidal *Salmonella* infection (Table 2.7).

Table 2.7: Level of awareness by total score

			Village ID				
Awareness_ Level	**Mafuluto**	**Malinzanga**	**Idodi**	**Mapogoro**	**Tungamalenga**	**Total count**	**Total Count (%)**
Low awareness	32(18.7)	35(20.5)	37(21.6)	31(18.1)	36(21.1)	171	79.5
High awareness	11(25.0)	8(18.2)	6(13.6)	12(27.3)	7(15.9)	44	20.5
Total	43(20)	43(20)	43(20)	43(20)	43(20)	215	100

Among the five villages, respondents from Mafuluto village had comparatively the highest level of awareness compared to the other villages. The minimum level of understanding was considered to be 41% score, hence any total score below 41% indicated that the respondent had low level of awareness and understanding about zoonoses and especially non-typhoidal *Salmonell*a infection (Figure 2.2).

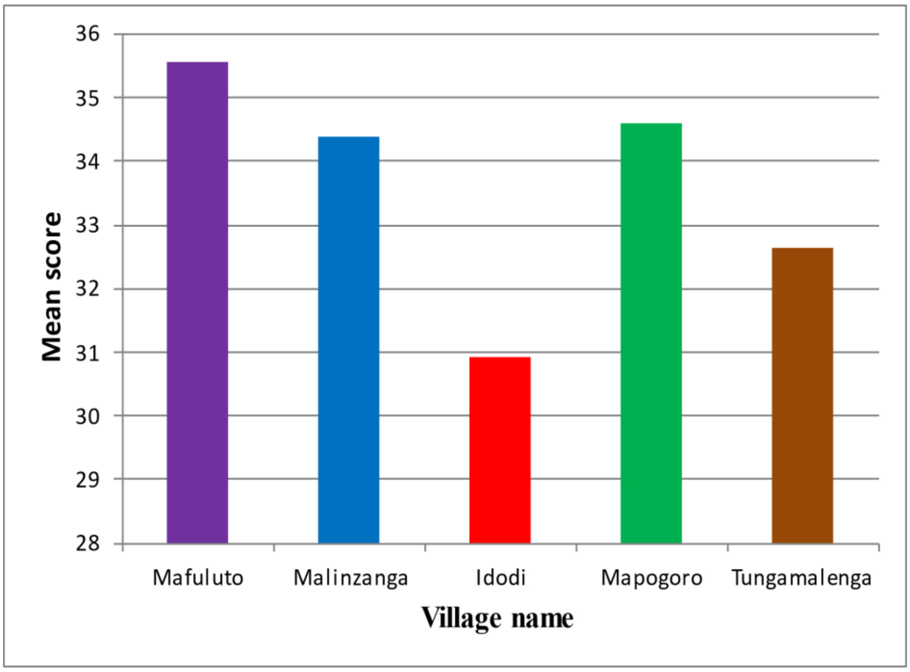

Figure 2.2: Mean score in level of awareness on zoonoses and non-typhoidal *Salmonella* infection in villages of Mlowa and Idodi wards

2.4 Discussion

The socioeconomic characteristics of respondents in this study showed that males were more engaged in animal husbandry (74.2%) compared to females (25.8%); The majority of the respondents were married (57.7%) with ages ranging from 30 to 50 years. These findings differ from those of [9], who reported that majority of respondents (77.6%) who were engaged in animal husbandry in Nigeria were females.

Most of respondents from this study were married, ages 30 to 50 years which shows that most people settle down and start rearing animals soon before or after marriage. The study also found that only 7% of the respondents were aged above 51. This could be because at this age a person declines in physical strength and becomes less involved in animal keeping.

In this study, livestock keepers' awareness on zoonotic infections was relatively high, where 100% of the interviewed livestock keepers were aware of zoonotic infections. This shows that the education about the existence of zoonotic diseases has well been provided to farmers hence increased precautions while handling diseased animals all over the society. These results agree with [10] who reported that the knowledge about zoonotic infections in Arusha and Tanga regions in Tanzania was quite high in 2010 (92%) among the smallholder dairy farmers. Among zoonotic infections, dengue fever (DF) was found to be known to many respondents as 98.1% of the respondents in this study seemed to have heard of dengue fever including its transmission and management. This was due to the fact that the coverage in mass media about dengue fever (DF) was high making the society aware of the disease.

The results closely resembled the findings by [11] where a study in Pwani region, Tanzania, showed that 97.7% of the people were aware of dengue fever (DF). However, this study revealed that none among the respondents was aware of Q fever, the disease or its symptoms. A study on the level of awareness of Q fever was conducted in Kajiado County in Kenya by [12] and it showed a relatively low level of understanding of 26.7%. This is probably because no effort has been put on educating the society about this zoonotic infection.

Regarding the trend of zoonotic infections, this study revealed that majority of the livestock keepers from the two wards involved in the study (79.5%) believed that the incidences of zoonotic infections have greatly decreased because awareness about the zoonoses and the control of zoonotic infections has been increasing over time. The results from this study resemble the findings that predicted that as the countries develop, most zoonoses will be minimized [13].

This study revealed that the awareness of livestock keepers of non-typhoidal *Salmonella* infection was low as only a small number of respondents (13.0%) were aware of non-typhoidal *Salmonella* infection based on the symptoms shown by the infected animals. The study revealed that majority of the respondents were unaware of NTS infection and most of other zoonoses, since only 20% of the respondents had a general understanding of zoonotic infections and non-typhoidal *Salmonella* infection in particular. A large number of people (87.0%) were unaware of non-typhoidal *Salmonella* infection; in fact 80% of the respondents had little understanding of zoonoses, especially the non-typhoidal *Salmonella* infection. This is said to be because of little efforts made on educating the farmers about non-typhoidal *Salmonella* infection. The awareness of non-typhoidal *Salmonella* infection is also low in some other parts of the world as it was reported by [14], where a study showed that only 40% and 30.2% of respondents from Miami and Los Angeles, U.S.A respectively, were aware of non-typhoidal *Salmonella* infection.

This study has indicated that for the past five years, domestic animals had suffered from non-typhoidal *Salmonella* infection and the livestock keepers noticed this as the symptoms were identified in such animals. The study showed that about 278 animals had suffered from non-typhoidal *Salmonella* infection from the year 2017 to 2021. About 501,700 Tsh. had been spent for the past five years to treat and control the non-typhoidal *Salmonella* infection in infected animals. This money could be put into better use for other economic activities.

The results from this study differ from those of [15], where the total annual *Salmonella* control costs in Denmark in year 2001 was U.S.$14.1 million. The amount used to control NTS diseases in the study site (Mlowa and Idodi wards) for five years was comparatively small because animal keepers lack adequate awareness. In spite of the fact that some efforts were put on the treatment of the diseased animals, a total of 161 died between 2017 to 2021 (Table 6). This shows the adverse economic impact of the NTS to animal keepers. The results from this study indicate that there had been no effective measures to control the disease. Also, livestock keepers had no knowledge about managing the disease, hence most animals died. The results agree with [16] who explained that most food borne diseases, especially *Salmonella* infection cost highly to control and if not well managed are fatal and causing big economic losses.

Conclusion

This study showed that community awareness on non-typhoidal *Salmonella* infection in the Ruaha National Park interface was low and the livestock keepers spent some resources in treating the animals with non-typhoidal *Salmonella* infection. Therefore, the non-typhoidal *Salmonella* infection caused significantly huge economic losses. The message of this study to the government is that non-typhoidal *Salmonella* infection causes increased mortality of infected animal hosts, it causes serious health problems to humans with compromised immune system and yet the livestock keepers are not sufficiently aware of it. This study and previous studies conducted in other parts of Tanzania recommend that non-typhoidal *Salmonella* infection must be taken as a serious problem, and more awareness is needed to communities around National Park Ecosystems and elsewhere in Tanzania on the existence of the infection in animals and potential transmission to humans.

If more investigations are carried out, awareness will be increased to the communities and safety intervention measures will be imposed to reduce the adverse economic impact of non-typhoidal *Salmonella* infection.

Recommendations

According to the findings from this study, we recommend the following;

i. More efforts should be made in creating awareness to the animal keepers about non-typhoidal *Salmonella* infection in order to minimize economic losses which are associated with this zoonotic infection.

ii. The Government of Tanzania through the wildlife and the public health sectors should invest in more research on zoonotic infections especially the ones which are less known to the people of Tanzania, such as non-typhoidal *Salmonella* infections, Q fever, Rift valley fever, Brucellosis and other zoonoses in order to obtain more information which will help on planning for disease management programs in the country especially at the wildlife and domestic interface.

iii. Livestock keepers should get trained on how they can improve the hygiene during animal rearing and animal product processing to minimize the zoonotic infections especially the non-typhoidal *Salmonella* infection.

Ethical Approval and Participants' Consent

Ethical clearance for this research was obtained from the Sokoine University of Agriculture Research and Publication Committee. The permit to conduct the research and collect samples from wild animals was obtained from the Tanzania Commission for Science and Technology with permit number 2021-237-NA-2021-086 while the permission to collect samples from domestic animals and data from respondents was obtained from the Iringa District Executive Director prior to data collection. Each respondent's consent was requested prior to participating in the study after the objectives and their benefits were well explained.

Competing Interests
The authors declare that they have no competing interests.

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CHAPTER THREE

Manuscript Two

3.0 Prevalence of Non-Typhoidal *Salmonella* Infection in Wild and Domestic Animals around Ruaha National Park Ecosystem, Tanzania

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Abstract
Non-typhoidal *Salmonella* infection is one of the zoonotic infections caused by Gram negative bacteria of the genus *Salmonella*, which belongs to the family *Enterobacteriaceae*. Non-typhoidal *Salmonella* infection is an important bacterial foodborne infection causing disease in animals and humans, particularly, immuno-compromised individuals. This study was conducted in 2021 and assessed the prevalence of non-typhoidal *Salmonella* infections in wild and domestic animals around the Ruaha National Park Ecosystem, Tanzania. A laboratory analysis of 215 fecal samples from wild and domestic animals was carried out to determine the prevalence of non-typhoidal *Salmonella* infection after the samples were enriched in Selenite F broth and incubated for 24 hours. The samples were primarily cultured on Salmonella Shigella agar (SSA), and in MacConkey agar then cultured on blood agar to observe swarming. Microscopy and Gram staining were conducted to check whether the suspected colonies were Gram negative since *Salmonellae* belong to this group. Confirmatory biochemical tests were conducted using the Triple Sugar Iron (TSI) test, IMViC tests, oxidase and catalase tests. Molecular confirmation was done by extracting bacterial DNA and then a conventional multiplex PCR was used for detection of the non-typhoidal *Salmonella* spp gene. Laboratory analysis revealed that non-typhoidal *Salmonella* infection was absent, with a prevalence of 0.00% in wild and domestic animals. Therefore, from the findings, we recommend more research on non-typhoidal *Salmonella* infection as well as other zoonoses in addition to increasing awareness to the communities on zoonoses. Awareness in the communities will help livestock keepers improve the animal rearing methods, hence reducing the transmission of zoonotic diseases, particularly the non-typhoidal *Salmonella* infection.

Key Words: Zoonoses, *Salmonella* spp., Non-typhoidal *Salmonellae*, Prevalence, Tanzania.

3.1 Introduction

Non-typhoidal *Salmonella* infection is one of the zoonotic diseases caused by the bacteria of the *Salmonella* group. It is transmitted from animals to humans, making it a big threat to the health and lives of humans around the globe. This disease is of great risks in areas with large concentrations of humans, animals and untreated manure and sewage contaminated surroundings (D'Aoust, 2007; Graham, 2002). In 2010, the World Health Organization estimated about 600 million cases of diseases were caused by contaminated foods of which about 350 million were due to pathogenic bacteria (Chlebicz & Śliżewska, 2018).

Salmonella bacteria belong to the family *Enterobacteriaceae*, they are non-spore-forming, facultative anaerobic, Gram-negative bacilli, motile by peritrichous flagella (Yoshikawa *et al.,* 1980). This pathogen may dwell in livestock such as poultry, cattle, and swine but are also found in wild animals, pets, fish, and rodents. Animals are often asymptomatic carriers of the pathogens, which they excrete in faeces, thus releasing the bacteria to the environment (Chlebicz & Śliżewska, 2018). *Salmonella* bacteria can survive for some time outside the human/animal host; as in water polluted with the excrement of carrier animals (Morpeth, 2009). Salmonellosis can affect animals and humans, as well as the wildlife.

Infection to humans usually occurs after eating meat or eggs and milk from domestic animals which have been contaminated with the bacteria. Non typhoidal *Salmonella* infection may present with one of several distinct clinical syndromes. However, common diseases by non-typhoidal *Salmonella* infection are: gastroenteritis, enteric fever, bacteremia, diarrhea and stomach cramps. These symptoms usually begin from six hours to six days after infection. Both focal infection and chronic state exist (Yoshikawa *et al*., 1980).

In Tanzania, previous studies have shown that invasive non-typhoidal *Salmonella* infection is an important cause of hospitalized febrile diseases among children**.** Invasive non-Typhi serotypes (NTS) salmonellosis is associated with a high risk of death (Mtove *et al.,* 2010). Lubote and colleagues reported that the prevalence of *Salmonella* species in some parts of Tanzania ranged from 0.17 to 28% (Lubote *et al.,* 2014).

The extent to which non-typhoidal *Salmonella* infection has spread between wild and domestic animals around the Ruaha National Park Ecosystem has not been explored. The present study, therefore, aimed to assess the prevalence of non-typhoidal *Salmonella* infection in wild and domestic animals in communities around this ecosystem.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted in the Ruaha National Park Ecosystem at Idodi division, in the Southern Highland Zone of Tanzania, Iringa region. Idodi displays a high interaction between domestic animals from the villages and wild animals from Ruaha National Park. Ruaha National Park is the largest wildlife park in Tanzania, which covers an area of about 20,226 square kilometers. It is located in the middle of Tanzania mainland about 130 kilometers from Iringa town. The Park is one of the Tanzania birds' paradise with more than 571 different species. It is also a place with magnificent mammals, reptiles and amphibians (Mtahiko, 2005).

The study was carried out in five villages namely; Idodi, Mapogolo, Tungamalenga, Malinzanga and Mafulutolo. These villages are located in two wards of Idodi division namely Idodi and Mlowa (Figure 3.1).

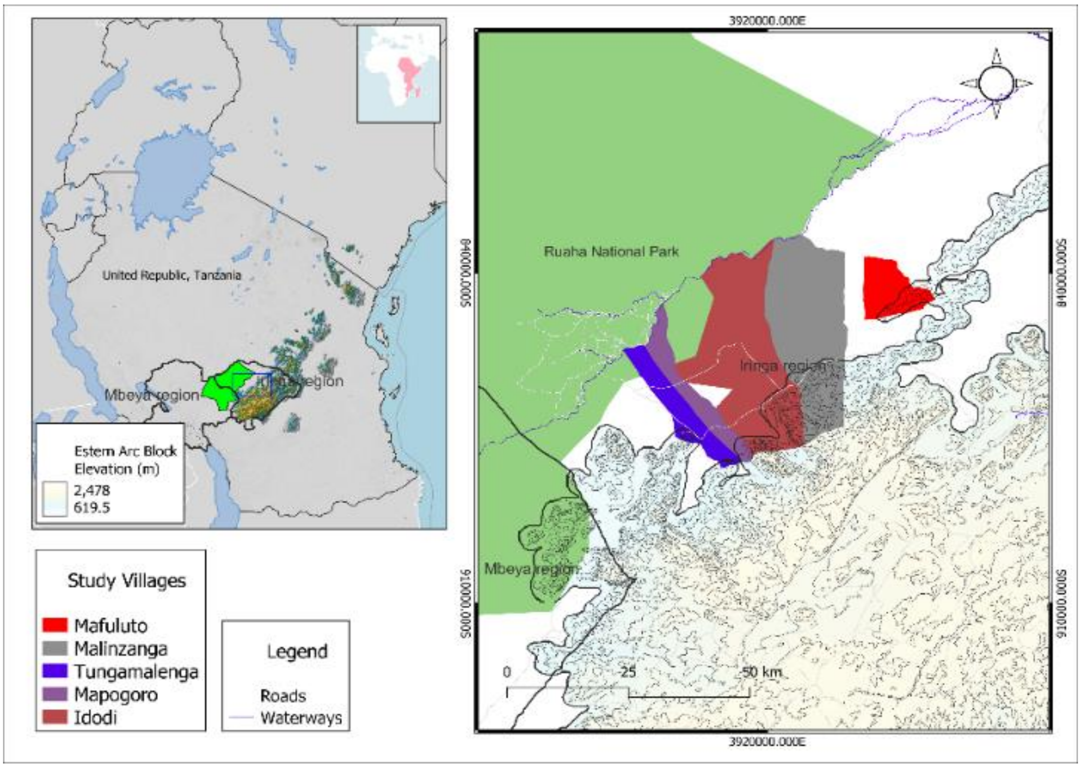

Figure 3.1: A map of Tanzania showing the Ruaha National Park Ecosystem and the study sites

3.2.2 Study design

A cross-sectional study design was conducted to determine the prevalence of non-typhoidal *Salmonella* infection in wild and domestic animals around the Ruaha National Park Ecosystem. Using an opportunistic random sampling strategy, five villages from Idodi division were selected purposively. The selection was based on the fact that Idodi division is located in the interface between the Ruaha National Park and human settlements and the other criteria was the prevalence of salmonellosis and other zoonotic diseases reported from previous studies.

3.2.3 Sample size, inclusion and exclusion criteria

The sample size was determined by using Cochran's formula n= $[Z^2P\ (1-P)]/d^2$ (Woolson *et al*., 1986) where, n =sample size; Z= statistic for level of confidence (1.96); P= prevalence (16.8% as described by Ngogo *et al.* (2020)); d= precision (5%). The sample size was 215 samples from wild and domestic animals.

Freshly dropped animal feces from wild and domestic animals found in the interface were collected for detection of non-typhoidal *Salmonellae* since it is in such locations where there is higher possibility of transmission of the bacteria between wild and domestic animals. Animals away from the interface were excluded from this study.

3.2.4 Sample collection and sampling strategy

Opportunistic random sampling technique was applied in the collection of the fecal samples from wild animals and selection of households for collecting fecal samples from domestic animals. 108 samples were collected from wild animals of which deer (39), elephants (22), monkeys (8), wild birds (14), wild pigs (2) and rodents (23). A total of 107 samples were collected from domestic animals of which chicken (26), cattle (24), goats (11), pigs (21), ducks (13), sheep (8) and guinea fowls (4).

3.2.5 Media preparation for bacterial isolation

Selenite F broth was prepared as per the protocol and used as the enrichment medium for activating the bacteria present in the fecal samples. The broth was cooled to 37^{o}C prior to its application for bacterial cultivation/growth. The medium used for primary isolation from the fecal samples was Salmonella-Shigella Agar (SSA) which was poured into the petri plates and then incubated at 37^{o}C overnight to check for sterility prior to inoculation.

3.2.6 Isolation and cultivation of non- typhoidal *Salmonella*

Cultivation of *Salmonella*

A swab with fecal sample was inoculated each separately into the freshly prepared Selenite F broth and marked appropriately. Then, these were incubated at 37°C overnight aerobically in a bacteriological incubator to activate the bacteria. Then the bacteria were inoculated onto SSA plate and incubated at 37°C overnight (Sarker *et al*., 2021).

Isolation of *Salmonella*

Salmonella culture on *Salmonella*-shigella agar

Isolation of *Salmonella* inoculum was done on SSA by streak plate technique to obtain isolated colonies (Sarker *et al*., 2021). The method was repeated until pure cultures containing single colonies were obtained.

Salmonella culture on blood agar

Blood agar was prepared according to the protocol. The petri plates with the BA were incubated overnight at 37^{o}C to check for sterility. After incubation, the suspect colonies were sub-cultured on the blood agar (3 quadrants) and incubated overnight at 37^{o}C.

Culture in MacConkey agar

MacConkey agar was prepared as per the protocol onto which the suspect *Salmonella* colonies were cultured. They were then incubated at 37^{o}C for 24 hours. (Dixon, 1961).

3.2.7 Identification and characterization of non-typhoidal *Salmonellae*

Salmonellae were identified based on their cultural characteristics, Gram staining property and by biochemical confirmatory tests as described by Sarker *et al*. (2021).

Gram staining and Microscopy

Salmonella colonies were stained using the Gram method (Packer & Merchant, 1967) to find out if the suspect colonies were Gram negative bacteria to which *Salmonella* spp. belong. Microscopy followed the staining by 100X magnification.

3.2.8 Confirmatory tests

Confirmatory tests were conducted as indicated below;

Triple Sugar Iron (TSI) test

Triple Sugar Iron (TSI) was used as the indicative test for *Salmonella* species and it was prepared according to protocol (Sagar, 2018; Sagar, 2019). The slants were incubated at 37^oC overnight to check for sterility then the suspect *Salmonella* colonies were inoculated onto the TSI slants and incubated at 37^oC for 24 hours.

IMViC tests

Indole, methyl Red (MR), Voges-Proskauer (VP) and Citrate Utilization tests were carried out. Each of the reagents for the IMViC test were prepared according to protocol, then the suspect *Salmonella* colonies were cultured and incubated at 37^oC for 24 hours (Yulistiani & Praseptiangga, 2019).

Oxidase test and Catalase test

Oxidase reagent and 3% H_2O_2 were prepared according to the protocol for oxidase test and catalase test respectively. The colonies were inoculated in each of them to observe the reactions. (Taylor & Achanzar, 1972).

3.2.9 Molecular methods for detection

DNA extraction

DNA extraction was performed as follows; each sample was diluted by mixing 2 colonies of culture with 200µl of Phosphate-buffered saline (PBS), in duplicates. The extraction process was done by the spin column technology using ZYMO DNA extraction kit (Zymo research, Quick-DNA Miniprep Plus Kit, Cat. No: D4068. Capacity 50 preps, U.S.A.) according to the manufacturer instructions. The extracted DNA was stored at -20^0C for further PCR analysis.

PCR

In this study, conventional multiplex PCR was used for detection of the non-typhoidal *Salmonella* species. The DNA extracted from the samples and the positive control was tested by PCR for carriage of genes for *Salmomella* spp., *S. enterica* ser. *typhi* and the two most common non-typhoidal *Salmonellae* which are *S. enterica* ser. *typhimurium* and *S. enterica* ser. *enteritidis*. The primer sequences and expected product sizes are shown in Table 3.1. Each multiplex PCR mixture in one reaction was prepared by using a total volume of 25µL containing 1µL forward primer, 1µL reverse primer, 2.5µL of 10X PCR buffer, 0.1µL U Taq Platinum DNA polymerase (Fermentas, Lithuania), 0.75µL of 50mM $MgCl_2$, 0.5µL of 10 mM dNTPs (Fermentas, Lithuania), 2µL DNA template and 17.15µL molecular grade water. The multiplex PCR was carried out through 30 cycles following a pre-heat step at 95 °C for 5 min. Each cycle consisted of denaturation at 95 °C for 60 sec, annealing at 57 °C for 1 min, and extension at 72 °C for 1 min. After the 30 cycles, samples were maintained at 72 °C for 10 min. *Salmonella enterica* subsp. *typhimurium* was included in each PCR assay as a positive control. The amplified DNA was separated by 2% agarose gel electrophoresis, stained with ethidium bromide and was visualized by UV transillumination (Ranjbar *et al.,* 2017).

Table 3.1: Primers used in this study (Ranjbar *et al.,* 2017)

Bacterial strains	**Target gene**	**Sequence of the primers**	**PCR Size (bp)**
Salmonella spp	*inv*A - secretory protein	F:5′-GTATTGTTGATTAATGAGATCCG-3′ R: 5′-ATATTACGCACGGAAACACGTT-3′	404
S. enterica serovar *typhi*	STY4669 - hypothetical protein	F:5′-TGTCCGCTGTCTGAAGTCATC-3′ R: 5′-ATCTCAGGCAAACTCACAAGGG-3′	489
S. enterica serovar *typhimurium*	STM0159 - restriction endonuclease	F:5′-ATGATGCCTTTTGCTGCTTT-3′ R: 5′-TCCCAGCTCATCCAAAAA-3′	224
S. enterica serovar *enteritidis*	SEN1383 - hypothetical protein	F:5′-TGTGTTTTATCTGATGCAAGAGG-3′ R: 5′-TGAACTACGTTCGTTCTTCTGG-3′	304

3.2.10 Data analysis

The prevalence of non-typhoidal *Salmonella* infection in wild and domestic animals was calculated by dividing the *Salmonella* positive samples by the total number of examined samples as described by Kelsey *et al*. (2000).

$$\text{Prevalence} = \frac{\textit{Salmonella}\ \text{positive samples}}{\text{Total number of samples examined}} \text{X}100\%$$

3.3 Results and Discussion

3.3.1 Results

Primary culture of isolates on SSA

In all 215 samples, there existed in primary cultures slight pink smooth colonies indicative of *E. coli* according to Islam *et al*. (2014). The *E. coli* were observed along with the *Salmonella* suspect colonies (black) as shown in (Figure 3.2).

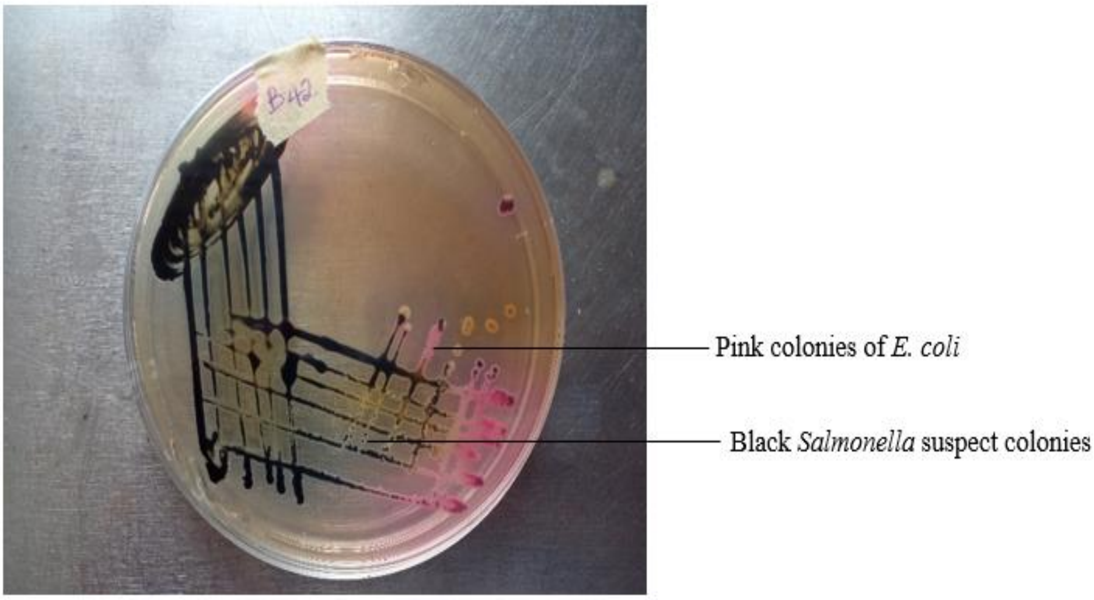

Figure 3.2: A plate showing the pink colonies of *E. coli* and black *Salmonella* suspect colonies (SSA)

***Salmonella* isolation in Salmonella Shigella Agar (SSA)**

Of the 215 samples collected from the wild and domestic animals and sub cultured on SSA for purification, 22 samples showed pure colonies with black centers indicating that the bacteria were producing Hydrogen Sulfide (H_2S). These were selected for further procedures as suspect *Salmonella* (Figure 3).

Figure 3.3: A plate showing the black *Salmonella* suspect colonies in SSA

***Salmonella* culture in blood agar**

On blood agar, out of the 22 suspect colonies cultured, five showed no swarming. The non-swarming colonies on petri plates were grayish white and grew well indicating that the colonies were not from other groups such as the *Proteus* spp. which usually show similar characteristics to *Salmonella* spp. on SSA, but have a swarming feature (Figure 3.4).

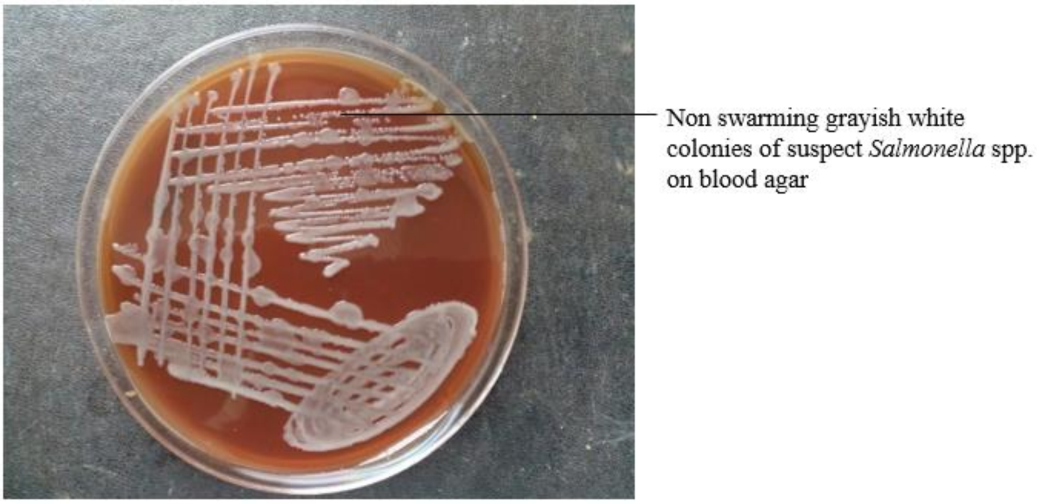

Figure 4: A plate showing non-swarming grayish white colonies of suspect *Salmonella* spp. on blood agar

Culture in MacConkey agar

Colourless and transparent colonies which did not alter the appearance of the medium were observed in the five *Salmonella* suspect samples (Figure 3.5).

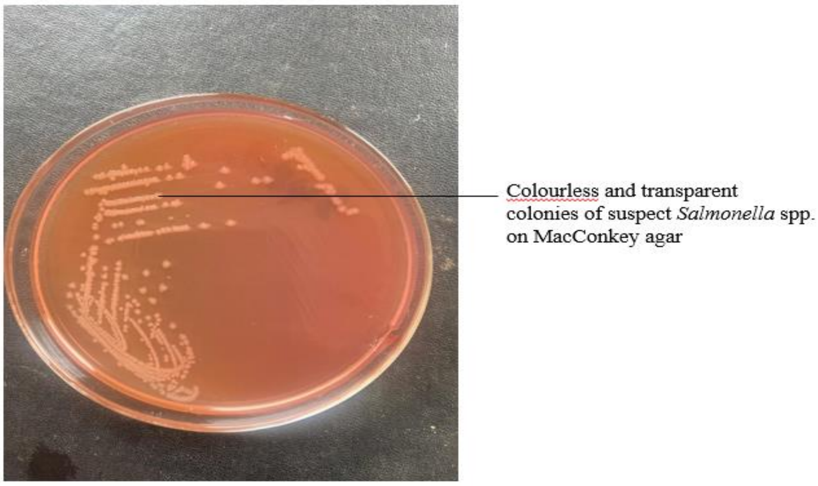

Figure 3.5: A plate showing the colourless and transparent colonies of suspect *Salmonella* spp. on MacConkey agar

Gram stain and microscopy

Gram staining of the five suspect *Salmonella* colonies showed pink colored rod-shaped bacteria, indicating Gram negativity. This confirmed that they were suspect colonies of *Salmonella* spp. (Figure 3.6).

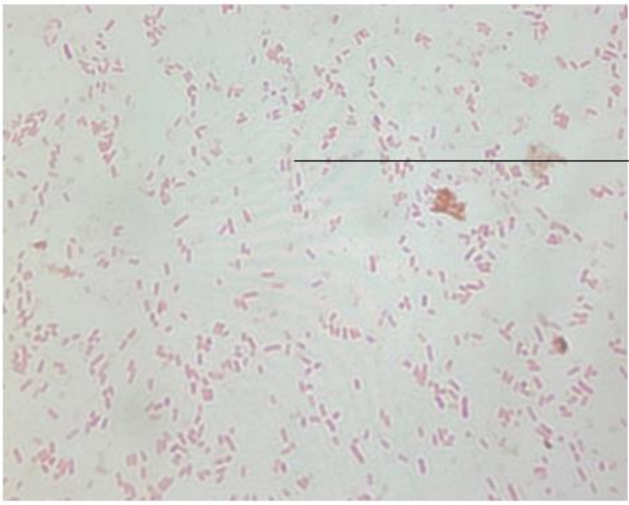

Figure 3.6: Gram negative *Salmonella* suspects under the microscope

Triple Sugar Iron (TSI) test

In a TSI test, the appearance of different colors in the slant and butt mean the presence of *Salmonella* species. The range of colours may be; red slant, black butt (H_2S) and gas + (Yulistiani & Praseptiangga, 2019; Sagar, 2018) or red slant, yellow butt, black butt (H_2S) and gas + (Sagar, 2019) or red slant, yellow butt and gas + (Islam *et al*., 2014; Rahman *et al*., 2019). The five samples tested presented a red slant, yellow butt, H_2S, gas positive (Figure 3.7).

Figure 3.7: TSI slants showing suspect *Samonella* spp.

IMViC tests
One of the five samples tested negative (-) for Indole test, positive (+) for Methyl Red (MR) test, negative (-) for Voges-Proskauer (VP) test and negative (-) for Citrate Utilization test. These results indicate the suspect *Salmonella* spp. (Figure 3.8).

Figure 3.8: IMViC test results

Oxidase test and Catalase test
The one suspect sample also tested negative (-) for oxidase test and negative (-) for catalase test.

Table 3.2: IMViC, oxidase and catalase test results for the suspect *Salmonella* spp.

Test / Sample	Indole	MR	VP	Citrate	Oxidase	Catalase	Result
B48	-	+	-	-	-	-	*Salmonella* spp. suspected

PCR results
The Positive Control (*Salmonella enterica* serovar *typhimurium*) indicated a band at expected base pair at genus level of *Salmonella* 404bp of the targeted gene invA- secretory protein. At the species level of *Salmonella enterica* serovar *typhimurium* a 224bp of the targeted gene STM0159- Restriction endonuclease was shown (Figure 3.9). However, the samples didn't indicate any amplification bands hence tested negative for *Salmonella* spp. (Figure 3.9).

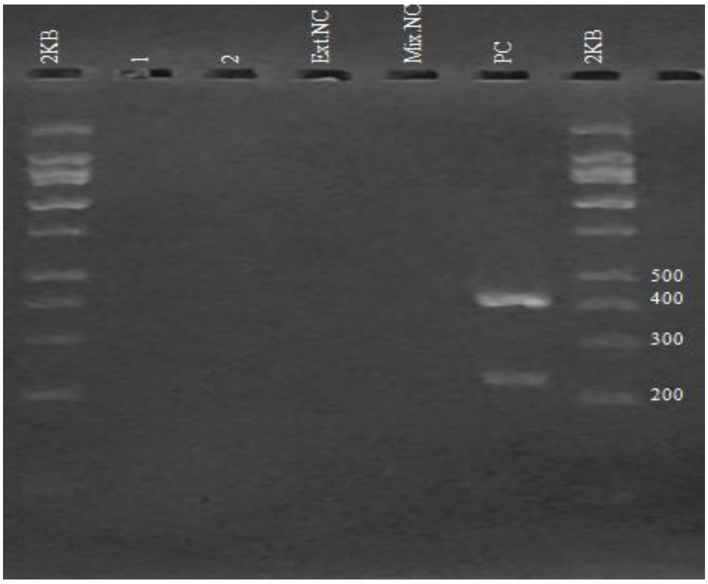

Figure 3.9: Multiplex PCR showing no amplification from the samples and showing amplification on the positive control (*Salmonella* enterica serovar *typhimurium*) DNA fragments from crude DNA extracts

Prevalence of non-typhoidal *Salmonella* infection in wild animals

The prevalence of non-typhoidal *Salmonella* infection in wild animals was calculated after the PCR confirmation. None of the 108 samples examined were positive for non-typhoidal *Salmonella* spp. (Table 3.3).

Prevalence of non-typhoidal *Salmonella* infection in domestic animals

In domestic animal samples, none of the 107 samples examined (0.00%) were positive for non-typhoidal *Salmonella* spp. (Table 3.3).

Table 3.3: Prevalence of non-typhoidal *Salmonella* infection in wild and domestic animals

Animals	**No. of samples examined (n=215)**	**No. of positive samples**	**Prevalence %**	**Overall prevalence %**
Wild animals	108	0.00	0.00	0.00
Domestic animals	107	0.00	0.00	

3.3.2 Discussion

Based on this study, NTS was not prevalent in the interface and this differs with a study that indicated a prevalence of NTS infection in the Southern highland to be 16.5% (Ngogo *et al*., 2020). Sarkar *et al*. (2021) reported that the general prevalence of *Salmonella enterica* serovar *enterica* in commercial chicken was 41% in Rajshahi district of Bangladesh. Another study conducted by Alali *et al*. (2012) in three regions of Russia showed that the overall prevalence of non-typhoidal *Salmonella* in chicken was 31.5%. This study showed that NTS was not prevalent and closely related to the findings from other parts of Southern highland zone of Tanzania and this could be because of the fact that there is variation in climatic conditions between the areas from where these studies were conducted (Tanzania Southern highland zone, Bangladesh and Russia). In the Ruaha National Park Ecosystem there are few animal keepers, with small flocks and hence implying reduced animal interaction therefore measures taken to control and combat the non-typhoidal *Salmonella* infection can be more effective compared to areas with many farmers keeping large animal flocks.

The findings from this study showed that non-typhoidal *Salmonellae* are associated with *E. coli* cells, similar to what was reported by Winfield & Groisman (2003). *Escherichia coli* are termed as ‘indicator’ organisms for non-typhoidal *Salmonellae* because they exist in the same environments, implying that usually the host environment provided adequately for the survival of the two species of microbes. Being in the same environment, *E. coli* and *Salmonellae* are perhaps commensals/mutualists rather than competitors.

None of the 108 samples collected from wild animals were positive for non-typhoidal *Salmonella* bacteria. This prevalence was low and agrees with the findings by Smith *et al*. (2002), where the prevalence in wildlife species in California, USA was found to be 4%. Furthermore, the findings of this study correspond with the findings of a study conducted by Parsons *et al*. (2010) who reported that the prevalence of non-typhoidal *Salmonella* was 4.2% in Australian wildlife. Findings in the reported studies indicate a lower prevalence of non-typhoidal *Salmonella* infection implying that there is reduced rate of spread of the bacteria among wild animals than domestic animals, perhaps because wild animals have a large space in the wilderness and that they can freely move from point to point without physical contact and contamination. Wild animals can feed and drink separately at any part of the wilderness, unlike the domestic animals which must share feeds and water such that contact with one another is inevitable.

None of the 107 analyzed samples from domestic animals (cattle, sheep, goats, chicken, ducks, guinea fowls, pigs) were found to contain any *Salmonella* spp. According to Eguale (2018), a study on non-typhoidal *Salmonella* serovars in poultry farms conducted in Central Ethiopia, the prevalence was 7.6%. Another study showed that the prevalence in domestic goats in Ethiopia was 3.86% (Tadesse & Gebremedhin, 2015). Tabo *et al*. (2013) indicated that the prevalence of NTS in poultry in the province of N’Djamena, Chad was 11.9%. The present study agrees with these studies which indicate low NTS prevalence in domestic animals. This suggests that the larger animal rearing grounds in African countries reduce significantly the number of animals which could suffer from the infection when a disease erupts as the animals seldom get in contact with others hence reducing the transmission rate of non-typhoidal *Salmonella* infection or any other infection compared to other regions like Bangladesh, Asia where farmers own large number of animals in small animal rearing grounds.

This study as well as other studies conducted in other areas suggest none or low prevalence of non-typhoidal *Salmonella* infection and if some efforts such as animal treatment programs and farmer awareness were added in controlling the non-typhoidal *Salmonella* infections then it could be possible to minimize the problem to a point where the domestic and wild animals will live free from non-typhoidal *Salmonella* infection.

3.4 Conclusion

This study revealed that there was no NTS infection in wild and domestic animals in Ruaha National Park Ecosystem at the time of the research. The contribution of this study to domestic and wildlife health is that, these animals in the interface at Ruaha National Park Ecosystem, especially Idodi division, are free from non-typhoidal *Salmonella* infection. However, more effort is needed on the investigation of more potential zoonotic microbial diseases in different national park ecosystems in order to create more awareness to the communities and introduce intervention measures to reduce the risks of zoonoses and especially the non-typhoidal *Salmonella* infection.

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CHAPTER FOUR

4.0 General discussion

This study showed no evidence of NTS infection in the interface. These findings are in disagreement with a study that indicated the prevalence of non-typhoidal *Salmonella* infection in the Southern highlands zone to be 16.5% (Ngogo *et al*., 2020). The findings in this study also disagree with the findings by Sarkar *et al*. (2021) who reported that the general prevalence of *Salmonella enterica* in commercial chicken was 41% in Rajshahi district of Bangladesh. Another study conducted by Alali *et al*. (2012) in three regions of Russia showed that the overall prevalence of non-typhoidal *Salmonella* in chicken was 31.5%. However, in Ruaha National Park Ecosystem there are few animal keepers with small herds hence implying that any measures taken to control and combat the non-typhoidal *Salmonella* infection should be effective with less transmission rate compared to areas with many farmers keeping large animal herds.

The study also revealed no non-typhoidal *Salmonella* infection in wild animals. These findings closely correspond with the findings by Smith *et al*. (2002) in California USA where the prevalence in wildlife species was found to be 4% and the findings by Parsons *et al*. (2010) who reported a prevalence of non-typhoidal *Salmonella* of 4.2% in Australian wildlife. The findings imply that there is a lower transmission of the bacteria among wild animals compared to domestic animals. This could be because wild animals have a large territory in the wilderness and can freely move from point to point without much physical contact that would lead to infection. Wild animals can feed and drink alone at any part of the wilderness unlike the domestic animals which must share any feeds and water available as well as get into contact with one another because of the small space in their housing.

According to Eguale (2018), a study on non-typhoidal *Salmonella* serovars in poultry farms conducted in Central Ethiopia reported a prevalence of 7.6%. Another study showed that the prevalence in domestic goats in Ethiopia was 3.86% (Tadesse and Gebremedhin, 2015). This was similar to a study conducted by Tabo *et al*. (2013) which indicated that the prevalence in poultry in the province of N'Djamena, Chad was 11.9%. This study agrees with studies in Chad and Ethiopia as all studies indicate low prevalence in domestic animals. This suggests that the larger animal rearing grounds in African countries make a smaller number of animals suffer from the infection when a disease erupts as the animals seldom get in contact with others hence reducing the transmission rate of non-typhoidal *Salmonella* infection as well as any other infections compared to other regions like Bangladesh, Asia where farmers own large number of animals in small animal rearing grounds.

The socioeconomic characteristics of respondents in this study showed that males were more engaged in animal husbandry (74.2%) compared to the females (25.8%). Also, the majority of the respondents were married (57.7%) with their ages ranging from 30 to 50 years, and these findings differ from the findings by Smith *et al*. (2010) who reported that majority of respondents (77.6%) who were engaged in animal husbandry in Nigeria were females. The difference between the findings from the two studies are mostly because societies divide roles differently where according to gender men suit to certain types of activities like animal keeping while women suit to activities like food traders based on the respondents age (30 to 50 years). It shows that most people settle down and start rearing animals shortly before or soon after marriage. Animal keeping can help the family get some income at any time of need. The study also found that only 7% of the respondents were aged above 51 years and this could be because at this age a person experiences decline in physical strength hence becoming less involved in animal keeping as well as other tough activities in general.

In this study, awareness of livestock keepers on zoonotic infections was high, where 100% of all the interviewed livestock keepers responded being aware of zoonotic infections. This shows that knowledge about the existence of zoonotic diseases has well been imparted to farmers who take precautions while handling diseased animals. These results agree with Swai *et al*. (2010) who reported that the knowledge about zoonotic infections in Arusha and Tanga regions in Tanzania was quite high (92%) among the smallholder dairy farmers. Among the zoonotic infections, dengue fever (DF) was found to be mostly known to many respondents as 98.1% of the respondents in this study seemed to have heard of dengue fever including its transmission methods and how to control the transmission. This was due to the fact that the coverage in mass media about dengue fever (DF) was high making the society aware of the disease. The results closely resembled to the findings reported by Kazaura (2020) where a study in Pwani region, Tanzania showed that 97.7% of the people were aware of dengue fever (DF). However, this study revealed that none among the respondents was aware of Q fever. A study on the level of awareness of Q fever conducted in Kajiado County in Kenya by Oboge (2018) showed a low level of understanding with the prevalence of 26.7%. This agrees with the results from this study. This is most probably because of the fact that less or no effort has been put on educating the society about this zoonotic infection.

Regarding the trend of zoonotic infections, we found that the majority of livestock keepers from the two wards involved in the study (79.5%) believed that the incidences of zoonotic infections have decreased as the awareness about the zoonoses and the control of zoonotic infections have increased over time. The results from this study resemble the findings that predict that in the next 20 years most zoonoses will be eliminated (Meslin, 1995). The implication from these findings is that efforts to ensure that societies are aware of zoonoses have been increasing lately resulting into a decrease in the occurrences of zoonotic infections.

This study revealed that the awareness of livestock keepers on non-typhoidal *Salmonella* infection was low as only a small number of respondents (13.0%) were aware of non-typhoidal *Salmonella* infection based on the symptoms shown by the infected animals. The study revealed also from the total score of the general understanding of non-typhoidal *Salmonella* infection that the majority of respondents were unaware of this zoonotic infection and most other zoonoses, since only 20% of the respondents seemed to have a general understanding of zoonotic infections and non-typhoidal *Salmonella* in particular. A large number of people (87.0%) were unaware of non-typhoidal *Salmonella* infection, 80% of the respondents had a low general understanding of zoonoses especially the non-typhoidal *Salmonella* infection and this is said to be because of little efforts made on educating the farmers about *Salmonella* infection. The awareness on non-typhoidal *Salmonella* infection is also low in some other parts of the world as it was reported by Beam *et al*. (2013) where a study showed that only 40% and 30.2% of respondents from Miami and Los Angeles, U.S.A respectively were aware of non-typhoidal *Salmonella* infection.

This study indicated that for the past five years, domestic animals have suffered from non-typhoidal *Salmonella* infection, based on the symptoms identified. The study showed that about 278 animals had suffered from non-typhoidal *Salmonella* infection from the year 2017 to 2021, where in 2017 a total of 21 animals suffered from non-typhoidal *Salmonella* infection, in 2018 a total of 66 animals suffered from non-typhoidal *Salmonella* infection, in 2019 a total of 73 animals suffered from non-typhoidal *Salmonella* infection, in 2020 a total of 110 animals suffered from non-typhoidal *Salmonella* infection and in 2021 when the study was being conducted a total of 8 animals were suffering from non-typhoidal *Salmonella* infection. About 501,700 Tsh. had been spent for the past five years to treat and control the non-typhoidal *Salmonella* infection in infected animals. This revealed that the presence of non-typhoidal *Salmonella* infection caused loss to the livestock keepers. The results from this study are quite different from the results reported by Wegener *et al*., (2003) where a total annual *Salmonella* control costs in Denmark in year 2001 was U.S.$14.1 million.

The amount used to control *Salmonella* diseases in the study site (Mlowa and Idodi wards) for five years was small and that was due to low awareness. In spite of the fact that some efforts were put on the treatment of the diseased animals, this study indicated that a total of 161 died in those five years where 18 died in 2017, 32 died in 2018, 38 died in 2019, 65 died in 2020 and 8 died in 2021. Death from NTS infection caused a loss of about 3,400,500 Tsh. from the year 2017 to 2020. Therefore, that non-typhoidal *Salmonella* infection had a significant negative economic impact.

The results from this study indicate that there had been no proper measures to control the disease, it also indicates that the livestock keepers had no knowledge about controlling the disease. The results agree with Todd (1989) who explained that most food borne diseases especially *Salmonella* infection cost a lot to control and if not well controlled then lead to big economic losses.

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CHAPTER FIVE

5.0 General Conclusion and Recommendations

5.1 Conclusion

This study revealed that non-typhoidal *Salmonella* infection in wild and domestic animals was not prevalent in the Ruaha National Park Ecosystem. This study also indicated that the livestock keepers had spent a lot of money for treating animals with non-typhoidal *Salmonella* infection before the year that this study was conducted and some of them lost their animals which died because of the infections, this indicated that the presence of non-typhoidal *Salmonella* infection caused economic losses hence negatively affected the economy of the livestock keepers in Idodi division. The contribution of this study to the animal health sector and to the wildlife sector is that, wild and domestic animals at the interface at Ruaha National Park Ecosystem, especially Idodi division are infected by non-typhoidal *Salmonellae* like any other susceptible animals in other ecosystems. Non typhoidal *Salmonella* infection, however, is dangerous when it comes to animal rearing and with the severe infection it causes, increased mortality rates to the infected hosts, but also causes serious health problems to humans with compromised immune system at any age. Considering this study and referring to previous studies conducted in other parts of Tanzania, non-typhoidal *Salmonella* infection has to be taken as a serious problem in order to bring more awareness to communities around National Park Ecosystems in Tanzania on the existence of non-typhoidal *Salmonella* infection in animals around their environments.

The expectation is that more effort can be added on investigation of more zoonotic infectious microbial species around national park ecosystems in Tanzania especially the Ruaha National Park Ecosystem as there is high interaction of wild and domestic animals at the interface. None of the known *Salmonella* species were found in this study but researches from other parts of the world like U.S.A and Ethiopia indicated that there is a number non-typhoidal *Salmonella* species which infect wild and domestic animals, so if more effort is put on the investigations, then different strains can be identified in different national park ecosystems and hence create more awareness to the communities. This was the first time that non typhoidal *Salmonella* infection was investigated in Mlowa and Idodi wards which are parts of the Ruaha National Park Ecosystem, hence there was limited reports for references and this challenge will be reduced if more research will be conducted on *Salmonella* infections in order to simplify the future research work.

5.2 Recommendations

According to the findings from this study, we recommend that the Government of Tanzania through the wildlife and the animal health sectors should invest in more research on zoonotic infections especially the ones which are less known to the people of Tanzania, such as *Salmonella* infections, Q fever, Rift valley fever, Brucellosis and other zoonoses which are not well known so as to increase the value of animals and animal products in national and international markets.

We also recommend that more efforts should be made in creating awareness to the animal keepers about non-typhoidal *Salmonella* infection in order to minimize economic losses which are associated with this zoonotic infection.

Until this moment, there is little information on *Salmonella* infection around Ruaha National Park Ecosystem and this made data collection difficult because the livestock keepers had little information about non-typhoidal *Salmonella* infection.

More studies on zoonotic infections are recommended to be conducted in order to obtain more information which will help on planning for disease management in the future. We recommend that the livestock keepers get trained on how they can improve the hygiene during animal rearing and animal product processing to minimize zoonotic infections.

APPENDICES

Appendix 1: Questionnaire administered in five villages of Idodi division for non-typhoidal Salmonella infection to livestock keepers of Ruaha National Park Ecosystem

QUESTIONNAIRE (DODOSO)

ASSESSMENTS OF PREVALENCE AND ECONOMIC EFFECTS OF NON TYPHOIDAL *Salmonella* INFECTION IN WILD AND DOMESTIC ANIMALS AROUND RUAHA NATIONAL PARK ECOSYSTEM

Section 1: Identification Particulars, Staff & Survey Time Details

1.1. DISTRICT	
1.2. DIVISION	
1.3. WARD	
1.4. VILLAGE	
1.5. NAME OF INTERVIEWEE (OPTIONAL)	
1.6. AGE OF THE OF INTERVIEWEE	
1.7. GENDER OF INTERVIEWEE	1 = Male, 2 = Female
1.9. MARITAL STATUS OF INTERVIEWEE	1= Married, 2 = Single, 3 = Widowed , 4= Divorced
1.10. NAME OF INTERVIEWER	
1.11. DATE OF INTERVIEW	
1.12. STARTING TIME	
1.13 . ENDING TIME	

Section 2: Household Enterprises, Income and Assets

2.1. Income from livestock enterprises during the **PAST 12 MONTHS**

Type	Number owned NOW	No. Sold (Past 12 Months)	Total revenue (Tsh)
Cattle			
Goat			
Sheep			
Chicken			
Duck			
Donkey			
Pig			
Milk			
Eggs			
Others			
TOTAL			

2.2. Income from livestock enterprises **before 2020**

Type	Number owned before 2020	No. Sold (before 2020)	Total revenue (Tsh)
Cattle			
Goat			
Sheep			
Chicken			
Duck			
Donkey			
Pig			
Milk			
Eggs			
Others			
TOTAL			

2.3. Income from wild animals and/or other wildlife products during the **PAST 12 MONTHS** (specify species/names).

Type/species	Number harvested	Total revenue (Tsh)

2.4. Income from wild animals and/or other wildlife products gained by the household **before 2020** (specify species/names).

Type/species	Number harvested before 2020	Total revenue (Tsh)

2.5. Other sources of income obtained in the past 12 months.

Source	**Quantity for Past 12 Months (specify units).**	**Total revenue (Tsh).**

Section 3: Views on Zoonotic Diseases

3.1. Have you heard of zoonotic diseases before? Yes/No

3.2. What are your views regarding the incidences of ZOONOTIC DISEASES in your area before and after 2020?
(1) Have decreased substantially; **(2)** Have decreased slightly; **(3)** Have remained almost unchanged; **(4)** Have slightly increased; **(5)** Have increased substantially.

3.3. What are the common ZOONOTIC DISEASES in your area which you are aware of?

Disease	**Put a tick where applicable (√)**
Brucellosis	
Bovine Tuberculosis	
Swine Flu	
Bird Flu	
Dengue Fever	
Q Fever	
Rabies	
Rift Valley Fever	
Anthrax	
Salmonella infection	
OTHERS (Name if any).............................	

Section 4: Views on Non Typhoidal *Salmonella* infection

4.1. Are you aware of non-typhoidal *Salmonella* infection? Yes/No

4.2. If yes, how did you know about it?

....

4.3. Have any animals from your household suffered from non-typhoidal *Salmonella* infection? Yes/No

4.4. If yes, how many animals/times became sick, how much money was spent for treatment and how much resources were lost due to the animals being sick and unable to produce or being used as intended?

Year	**No. Of cases or animals**	**Total cost of treatment**	**Resources and amounts lost**	**Estimated costs of resources lost**
2017				
2018				
2019				
2020				
2021				

Appendix 2: Participant Consent Form and Privacy Notice

Participant Consent Form and Privacy Notice
Interviewee:
………………………………………………………………………………………………
Organisation:
………………………………………………………………………………………………
Interviewer:
…………………………………………………………………………………………………
Date: ……………………………………………………………………………………….
Location: ………………………………………………………………………………….

1. Thank you for agreeing to be interviewed as part of the **TRADEHUB** project. The aim of this project is to address the intractable challenge of how to eliminate the negative impacts of trade in wildlife and agricultural commodities on people and ecosystem.
2. The high level project aims are; To map relevant trade policies in wildlife- and commodity-exporting countries, value chain structures, strategies, agreements, protocols, demand and supply balance sheets for modeling of economic impacts. Also to analyse international trade governance and performance, quantification of trade values and factors that influence trade both in the supply side and demand side
3. The aim of the data collection is to find out about the incidences of zoonotic diseases, particularly non typhoidal *Salmonella* infection and how they have affected the economy in your locality in the past years (before 2020). The aim is to find out whether *Salmonella* infection has had any economic effects in your locality and to find out whether the residents of your locality are aware of this zoonotic infection.
4. Your interview will be conducted via **questionnaire survey** and, only with your permission, will the report be recorded with notes written.

PRIVACY NOTICE

5. Your data will be used by **SOKOINE UNIVERSITY OF AGRICULTURE, SCHOOL OF AGRICULTURAL ECONOMICS AND BUSINESS STUDIES (SAEBS), P. O. BOX 3007, CHUO KIKUU-MOROGORO, TANZANIA. Email; saebs@sua.ac.tz Phone; +255(023)2603415.**

The personal data we would like to collect from you is:

- Name
- Gender
- Age
- Permanent address

6. For the purposes of this project, we will only store and share your personal data as follows:

   a. The data storage system will be the TRADE Hub SharePoint and these data will be password protected.

The data sharing will be; **Restricted groups access**, where only individuals belonging to the TRADE Hub project will have access to the data through a password and user names system.
b. Any information obtained during these interviews will be deleted within 6 months following the completion of the project

7. You have the right to withdraw from this interview or to request your data to be removed from the process at any time.

Please confirm that:

1. You have read and understood the above terms YES/NO (*please delete as appropriate*)
2. You agree, your interview to be recorded YES/NO (*please delete as appropriate*)
3. You would like your input into this process to be anonymous YES/NO (*please delete as appropriate*)

Under these circumstances, you agree to participate in the research:
Signature:
……………………………………………………………………………………………
Name (please print):____
Date:__________________________