EPIDEMIOLOGY OF *EDWARDSIELLA* INFECTIONS IN FARMED FISH IN MOROGORO, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HEALTH OF AQUATIC ANIMAL RESOURCES OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

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ABSTRACT

A cross sectional study was undertaken from November 2016 to April 2017 to find out whether *Edwardsiella* infections exist in farmed fish in Morogoro. The prevalence of infection, risk factors and fish haematological parameters were established. A total of 270 fish were sampled from 24 ponds. Each fish was clinically examined and aseptically swabs of kidney, liver and spleen and pond water were collected for bacteriology. Bacteria were cultured onto Tryptic soya agar and Salmonella-Shigella Agar. Colony morphology and biochemical tests were used for phenotypic identification of the bacterium and confirmed by conventional PCR through detection of *gyr*B, IRS and IVS in the 23S rRNA gene for *E. tarda* and *E. ictaluri,* respectively. Presumptive results identified 14 Gram-negative, rod-shaped similar biochemically with *Edwardsiella* bacterial isolates. Out of 14 only four isolates amplified the *gy*rB gene using specific *E. tarda* primers. Overall, the prevalence of *E. tarda* in the study area was 1.48% (n=270). *Clarias gariepinus* was at a higher risk of acquiring *E.tarda* infection than *Oreochromis niloticus* ($P < 0.05$). Since the bacterial prevalence was low, it was not possible to establish the correlation with hematological parameters. In addition, there was no association between water quality and the prevalence of *E. tarda* infections. For the first time, this study isolated and confirmed the occurrence of *E. tarda* pathogens in farmed fish in Morogoro, Tanzania. However, more work on virulence studies on *E. tarda* isolates need to be done. The establishment and implementation of biosecurity measures to prevent disease outbreak in fish farms is also recommended taking into account its zoonotic potential.

DECLARATION

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

_____ _____

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DEDICATION

This dissertation is dedicated to my beloved parents, my father the late Erick Mkemwa and my mother Rozaria Bakari

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LIST OF ABBREVIATION AND SYMBOLS

bp	Base pair
<,>	Less than, Greater than
µl	microliter
dl	decilitre
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
DO	Dissolved Oxygen
EDTA	Ethelyne Diamine Tetra Acid
FSDP	Fisheries Sector Development Programme
g	gram
IRS	Inter-Ribosomal Spacer
IVS	Intervening sequence
LAMP	Loop Mediated isothermal Amplification
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume.

NCCL	National committee for clinical laboratory standard.
NSSGRP	National Strategy for Growth and Reduction of Poverty.
PCR	Polymerize chain reaction
PCV	Packed cell volume
RBC	Red blood cell
SSA	Salmonella-Shigella Agar
Taq	*Thermus aquaticus*
TFAR	Tanzania Fisheries Annual Statists report
TSA	Tryptic Soya Agar
UV	Ultra-Violent light
WHO	World Health organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Aquaculture in Tanzania mainland is dominated by freshwater fish farming whereby fish farmers practice both extensive and semi-intensive farming involving an integrated farming with livestock (FAO, 2005; FSDP, 2010). *Oreochromis niloticus* (Nile tilapia) is the commonly farmed fish species. This is probably because of the incredible adaptability of the fish to a wide range of environmental conditions (Kaliba *et al*., 2006; Limbu *et al*., 2016). Their ability to thrive and reproduce in different environmental conditions has made the fish to become the first choice in many parts of Tanzania. *Clarias gariepinus* (African catfish) is another commonly farmed species in the country. The species is usually mixed with tilapia to control the population of the latter (FSDP, 2010; Limbu *et al*., 2016).

Aquaculture is estimated to produce about 2,676 tons of fish annually, which is equivalent to one percent of the total fish produced in Tanzania (TFAR, 2014). Since aquaculture is still at a subsistence level, its contribution to national food security and economic development is still insignificant. However, its benefits to the community are through the provision of animal derived protein, employment and a source of income to farmers (FAO, 2005).

Global declined fish catches from natural water bodies coupled with growing demand for animal protein and low national fish consumption rate which is about 8 kg per capita being below the FAO recommended level of 12.5 kg per capital

annually (FSDP, 2010), have resulted into strengthening of the strategies for boosting aquaculture production by the governments of East African countries. These strategies aim at filling the growing gap of production from capture fisheries (Kpogue *et al*., 2013; Kashindye *et al*., 2016). As a country, Tanzania has great potentials for aquaculture expansion and development due to availability of diversified species in the wild, which are suitable for culture and raw material for fish feed production (FSDP, 2010). The Government of Tanzania, through the National Strategy for Growth and Reduction of Poverty (NSSGRP II) has initiated the transformation of the aquaculture sub-sector into an intensive production system (FSDP, 2010).

In spite of that efforts, the sub sector is facing several challenges which include, limited quantity and quality seeds, limited feeds, and prevalence of fish diseases (Shoko *et al*., 2011; Mathew *et al*., 2014). Fish diseases limit the performance of both freshwater and marine fish farming in many parts of the world (Subasinghe, 2005; Murray and Peeler, 2005). Ineffective biosecurity measures among fish farms make the control of fish diseases difficult (Noga, 2010; Austin and Austin, 2014). The interaction of many factors and pathogens have resulted to the emergence of diseases in the freshwater fish farms (Mathew *et al*., 2014) and among the pathogens, bacteria are the most common causing major losses to fish industry worldwide (Camus *et al*., 1998; Mohanty and Sahoo, 2007).

Several species of bacteria have been isolated from fish in Africa, particularly in East Africa from fish farms with disease outbreaks. The commonly reported bacterial species are, Aeromonads, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*,

Mycobacterium and *Streptococcus* (Akoll and Mwanja, 2012). Although *Edwardsiella* species have been reported to occur in East Africa, the epidemiological information is limited. *Edwardsiella* species are important opportunistic pathogens in aquaculture which cause high mortalities in farmed as well as in wild fish worldwide (Sechter *et al*., 1983; Park *et al*., 2012). The disease infection in fish farms has been attributed to environmental stressors such as high water temperatures and poor pond management practices (Park *et al*., 2012).

Edwardsiella tarda and *E. ictaluri* are two species of genus *Edwardsiella*, commonly identified as pathogenic in both fresh and marine water, and cause diseases in various fish species of different age groups (Subasinghe, 2005). Fish infected with *Edwardsiella* species may present various clinical signs and pathological lesions such as abnormal swimming, anorexia, loss of skin pigmentation, exophthalmia, opacity, swelling of the abdominal surface, and haemorrhage in fin and skin depending on the species involved and the severity of the infection (Buller, 2014). Importantly, *E. tarda* has been reported to be zoonotic when coming in contact with human beings (Gormaz *et al*., 2014). It is important to establish epidemiological data of the disease in Tanzania. The data would be useful in the planning of its control measures in the country.

1.2 Problem statement and study justification

Aquaculture has been growing in Tanzania especially at small scale. However, these developments are undermined by the occurrence of fish diseases. There are few reports of the occurrence of disease and infection in fish farms around Morogoro Region; but the aetiology is unclear. Bacterial infections are common and where

studies have been conducted, *Edwardsiella* species is reported to be the frequent cause of the problem in fish farming (Centers for Epidemiology and Animal Health, 1995). Increased movement of live fish as seeds and the infectious nature of *Edwardsiella* have led to epizootics in different tropical farmed fish in many parts of the world. However, there is limited information about the status of the *Edwardsiella* infection in farmed fish in Morogoro, Tanzania. The occurrence of this bacterial infection elsewhere has been influenced by factors such as poor water quality and poor nutrition. These factors have not been investigated in Morogoro fish farms. Understanding the epidemiology of the disease and molecular characteristics of *Edwardsiella* organism would enable development of protection measures against the disease. This study provides baseline information on the status of *Edwardsiella* infections in farmed fish. This information would be useful for planning the prevention and control strategies against the disease in Tanzania.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to establish the prevalence and associated risk factors of *Edwardsiella* infections in *O. niloticus* and *C. gariepinus* farmed in Morogoro, Tanzania.

1.3.2 Specific objectives

The specific objectives of this study were;

i. To determine the prevalence of *Edwardsiella* infections in *O. niloticus* and *C. gariepinus* farmed in Morogoro, Tanzania;

ii. To assess the risk factors associated with *Edwardsiella* infections in farmed *O. niloticus* and *C. gariepinus* in Morogoro, Tanzania;

iii. To determine hematological parameters of the infected *O. niloticus* and *C. gariepinus* farmed in Morogoro, Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Status of fish farming in the world

Worldwide, aquaculture is growing faster than any other food production sectors, and this is often referred to as the blue revolution (Ashley, 2007; FAO, 2016). Meanwhile, significant expansion of the traditional land-food production systems such as crop cultivation and livestock farming is limited by the fact that the earth has only 30% land coverage (Peeler and Taylor, 2011). According to the state of world fisheries and aquaculture (SOFIA) report of 2016, a decline in capture fisheries has stimulated aquaculture growth worldwide (FAO, 2016).

The reported aquaculture growth has made the supply of fish for human consumption to outpace population growth in the past five decades and has doubled per capita intakes of fish from 10 kg per capita in 1960s to 20 kg per capita in recent years (FAO, 2016). China remains the leading nation for aquaculture, while in Africa, the industry is growing by 11.7% every year led by Egypt and Nigeria followed closely by sub-Saharan African countries (Peeler and Taylor, 2011; FAO, 2016). Sub-Saharan Africa's vast inland waters and coastlines present a largely untapped opportunity for aquaculture. The level of intensification in aquaculture and the use of non-native species have increased and this has created various stressors resulting in the emergence and spread of new pathogens and the occurrence of the disease (Shah *et al*., 2012).

2.2 Fish farming in Tanzania

In Tanzania, aquaculture is still largely a small-scale enterprise (FAO, 2005; Shah *et al*., 2012). According to TFAR (2014), aquaculture contributed 2,676.67 metric tons which is equivalent to 1% of total fish produced, while capture fisheries contributed 367,854 metric tons. In general, Tanzania fisheries sector is estimated to contribute 1.4% to the Gross Domestic Product (GDP) (TFAR, 2014). The national fish consumption is about 8 kg per capita which is still below the FAO recommended rate of 12.5 kg per capita annually. The existing gap of fish demand has been minimized by the importation of fish into the country (Kpogue *et al*., 2013).

Integrated freshwater fish farming, which is mainly characterized by small sized earthen ponds, is still the most commonly practiced form of aquaculture in the country. Farmers practice both monoculture and polyculture fish farming systems (FAO, 2005). Commonly farmed fish species are *O. niloticus* and *C. gariepinus* (FAO, 2005; Mathew *et al*., 2014). Integrated aquaculture is a traditional practice by small-scale farmers elsewhere in the world (Li *et al*., 2017). In this system, the wastes of the land animals and crops (manure and feed wastage) are used by fish directly or indirectly to stimulate growth of green algae which provides natural feeds for fish (Abdel-Hakim *et al*., 1999; FAO, 2005). This decreases the production cost of fish farming considerably (Abdel-Hakim *et al*., 1999). Sometimes, few supplements such as low quality feed including cereal bran, greens and table remains are applied (Mathew *et al*., 2014). Although integrated farming systems are sustainable in many ways, they may also transmit faecal pathogens and lead to the accumulation of antimicrobials and other drug residues originating from livestock (Shah *et al*., 2012; Keremah and Inko-Tariah, 2013).

Tanzania is likely to establish intensive aquaculture in the near future due to the availability of necessary potentials for aquaculture development such as diversified fish species in the wild which are suitable for fish farming and availability of raw materials for fish feed production (TFAR, 2014). The declining catches of capture fisheries in Lake Victoria and other natural water bodies and the growing demand for protein from fish have been the drivers in strengthening strategies of improving aquaculture production to fill the deficit (Kashindye *et al*., 2015). The Government, through different frameworks such as NSSR PII and Rural Development Strategy (RDS) of 2003 has initiated transformation of the sector from subsistence into commercial based (FSDR, 2010).

2.3 Challenges of fish productions in Tanzania

Like any other sector in Tanzania, fish farming face challenges such as slow adoption of appropriate technologies, including biotechnology and bio-safety, inadequate extension services, diseases and limited capacity in the diagnosis of fish diseases, insufficient infrastructure, low managerial farm skills, limited availability of quality fish seeds, fish feeds (FASR, 2010). These challenges make fish vulnerable to disease causing agents. Several parasites have been reported to infest farmed fish and wild populations (Mwita and Nkwengulila, 2008; Mathew *et al*., 2014). The fish infected with these parasites become weak and susceptible to opportunistic bacterial pathogens which are present in the water resulting to considerable deaths (Chitmanat *et al*., 2005). Also, several other pathogens such as fungus, bacteria and non-infectious conditions have been associated with morbidities and mortalities in both wild and farmed fish (Shah *et al*., 2012; Mgode *et al*., 2014; Moremi *et al*., 2016).

2.4 Bacterial diseases in Aquaculture

Bacteria are common pathogens of farmed fish, and when outbreaks occur, they cause major losses to aquaculture as well as mortalities in wild populations of fish (Almaw *et al*., 2004; Buller, 2004; Noga, 2010). Motile Aeromonads and *Edwardsiella* species are among the most common opportunistic bacteria causing fish diseases in freshwater (Mohanty and Sahoo, 2007), as they are abundant, widely distributed and affecting a wide range of fish species in freshwater and marine environment (Almaw *et al*., 2004; Mohanty and Sahoo, 2007; Pridgeon, 2012).

In Africa, particularly in East Africa, the major bacterial infections among tropical fish are caused by species of *Aeromonas*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Mycobacterium* and *Streptococcus* (Akoll and Mwanja, 2012). Although little research has been done in Tanzania, several species of bacteria have been reported including *Leptospira, Proteus, Aeromonads, Acinetobacter baumannii, Pseudomonas fluorescens,* and *Enterobacter species* (Shah *et al*., 2012; Danba *et al*., 2014; Moremi *et al*., 2016). Therefore, the transformation of subsistence aquaculture into commercial production increases the risks of bacterial infections due to increased deterioration of aquatic environment (Akoll and Mwanja, 2012).

Generally, epidemiological studies which have been carried out so far have covered the incidence/prevalence, distribution, risk factors and possible control of diseases in a population. For proper control of fish pathogens in aquaculture, there is a need of epidemiological studies aided with molecular techniques to explore the conditions under which putative pathogens spread between wild and farmed aquatic animals (Peeler and Taylor, 2011). This would enable evidence based biorisk planning for disease control in aquaculture (Subasinghe, 2005).

2.5 Predisposing factors for bacterial disease outbreaks

Bacterial disease outbreak is influenced by several factors that act jointly and whose combination behind the emergence of each disease is unique; though various common factors such as stressors are apparent (Woo and Bruno, 2011). Stress plays a key role in the outbreaks of fish disease caused by opportunistic bacteria (Oliva-Teles, 2012), which are most prevalent in aquatic environment (Cunningham *et al*., 2012; Buller, 2014). In aquaculture, stress is caused by fish handling, poor diet, poor water quality, overcrowding, and water temperature fluctuations (Woo and Bruno, 2011). Mixed-age fish farming or stocking young naive fish in a pond with carrier older fish and polyculture may play a key role in the spread of bacterial disease (Peeler and Taylor, 2011; Cunningham *et al*., 2014). The use of untreated surface water in the pond poses a risk of pathogens introduction to farmed fish (Oidtmann *et al*., 2013). In addition, the use of animal fresh manure in the fish pond, introduces the disease causing pathogens such as *E. tarda* and *Salmonella* species (Li *et al*., 2017). Increased aquatic animal movements for ornamental trade, food, and aquaculture across international terrestrial borders present an important way of pathogens transmission.

2.5.1 *Edwardsiella* infections

Edwardsiella infections are caused by bacteria from the family *Enterobacteriaceae* (Reichley and Ware, 2015) and genus *Edwardsiella* which consists of four species namely *E. tarda*, *E. hoshinae, E. piscicida*, and *E. ictaluri* (Park *et al*., 2012). The genus *Edwardsiella* is characterized by facultative anaerobic, Gram negative, oxidase-negative, catalase-positive, and is relatively inactive in terms of

carbohydrate metabolisms (Table 1) (Wang *et al*., 2011). The presence of two ribosomal gene clusters located in tandem to one another, the inter-ribosomal spacer (IRS) differentiates them from other members of the Enterobacteriaceae (Williams and Lawrence, 2010). However, within the genus *Edwardsiella*, species are differentiated based on their biochemical and physiological characteristics, natural habitats, and pathogenic properties (Mohanty and Sahoo, 2007).

Edwardsiella ictaluri has an intervening sequence (IVS) in the 23S *r*DNA gene which is absent in other species (Williams *et al*., 2008). Unlike the other three taxa within the genus, *E. tarda* is known to be a versatile pathogen with a wide ecological niche and infected wide range of host including various species of fish and other marine fauna, reptiles and terrestrial mammals including humans (Alcaide *et al*., 2006; Woo and Bruno, 2011). In contrast to *E. tarda*, *E. hoshinae* is found in a relatively few ecological niches such as birds, reptiles, and water. Also *E. hoshinae* has been isolated from human faeces but its role as a human or animal pathogen has not been established (Stock and Wiedemann, 2001; Woo and Bruno, 2011; Buller, 2014). *Edwardsiella ictaluri* has a low optimal growth temperature and has been predominantly isolated from channel catfish (Menanteau-Ledouble and Lawrence, 2011), which causes fatal systemic infections known as enteric septicaemia (Williams *et al*., 2008). Human infections due to *E. ictaluri* have not been reported (Alcaide *et al*., 2006).

2.5.2 Source of infection and transmission of *Edwardsiella*

Introduction of infection to a clean farm may be associated with introduction of new asymptomatic infected live fish, or animal faeces with bacteria, contaminated feeds or water (Sechter *et al*., 1983; Park *et al*., 2012; Oidtmann *et al*., 2013). Transmission of *Edwardsiella* bacteria between fish in the farm is from faecal shedding from infected fish or from the carcasses of a dead fish (Park *et al*., 2012; Hirono, 2013). Vertical transmission from infected brood stock to fry has not been demonstrated (Park *et al*., 2012). Ciliated protozoans such as *Trichodina* and *Tetrahymena pyriformis* can act as a vector for *E. tarda* transmission to farmed fish (Woo and Bruno, 2011).

Table 1: Biochemical characteristics of *Edwardsiella* species

Characteristics	**Reaction**			
	E. tarda	***E. ictaluri***	***E. hoshinae***	***E. piscicida***
Catalase	+	+	+	+
Voges-Proskauer	-	-	±	-
Methyl red	+	_	+	+
Indole production	+	-	±	+
H2S production	+	-	-	+
Lysine decarboxylase	+	-	-	+
Ornithine decarboxylase	+	-	-	+
Inter-ribosomal spacer (IRS)	+	+	+	+
Intervening sequence (IVS)	_	+	_	_
Citrate (sodium)	-	-	-	_
Urease	_	_	_	_
Fermentation/glucose				+
Motility	+	_	+	+
Growth at 40oC	+	_	-	_

2.5.3 Pathogenesis, clinical signs and pathological lesions

The pathogenesis of most species of *Edwardsiella* bacteria is multifactorial. The mechanisms are poorly understood, and the site of attachment and penetration are not known (Mohanty and Sahoo, 2007; Park *et al*., 2012), although the intestine and abraded skin are the most likely sites for penetration of the bacterium (Woo and Bruno, 2011; Buller, 2014). *Edwardsiella tarda* can cross the intestinal epithelium, enter the blood stream and migrate to reach the kidneys (Park *et al*., 2012). Its virulence factors such as siderophores, cell adhesion and hemolysin have been described (Pressly *et al*., 2005; Li *et al*., 2011) while the nares are the primary site for of *E. ictaluri* invasion (Woo and Bruno, 2011; Buller, 2014).

Fish infected with *Edwardsiella* species show variable clinical signs such as abnormal swimming behaviour, spiral movement and floating near the water surface. Other signs include anorexia, loss of skin pigmentation, exophthalmia, opacity, swelling of the abdominal surface, petechial haemorrhage in fin and skin, and rectal hernia (Iregui *et al*., 2012; Park *et al*., 2012). Internally, watery and bloody ascites and congested liver (Abraham *et al*., 2015), spleen, and kidney are commonly observed (Wyatt *et al*., 1979; Alcaide *et al*., 2006; Park *et al*., 2012). There are no clear pathognomic signs on infected fish, except in *E. ictaluri*, where ulcers on the top of the head and between the eyes of catfish "hole-in-the-head" have been reported (Williams *et al*., 2008).

Haematological parameters are important health indicators for fish (Stacy *et al*., 2014). Fish haematological parameters can be influenced by several factors such as age, sex, nutrition, breed, diseases, and pond environment (Weiss and Warddrop,

2010; Thrall *et al*., 2012). Haematological parameters can be useful in the detection of diseases affecting the cellular components of blood (Robert, 2012; Thrall *et al*., 2012). *Edwardsiella* infection also affect heamatological parameters of the fish by causing the reduction in haematocrit values, heamoglobin, erythrocytes count, total plasma proteins, and an increase in the total leukocytes count (Mohanty and Sahoo, 2007). Thus, assessing heamatological values is a valuable tool for the diagnosis of fish diseases (Hrubec *et al*., 2000).

2.5.4 Diagnosis of *Edwardsiella* infections

Edwardsiella infection in fish, like any other bacterial infection can be diagnosed by several methods including clinical signs, histopathology, haematology, bacterial isolation and biochemical characteristics (Mohanty and Sahoo, 2007; Park *et al*., 2012; Hrubec *et al*., 2000; Hemraj *et al*., 2013). Several serological techniques including agglutination tests, Enzyme Linked Immunosorbent Assays (ELISA), and Fluorescent Antibody Techniques are useful for the diagnosis of *Edwardsiella* infection (Woo and Bruno, 2011). Following recent technological advancement, PCR-based methods and the loop mediated isothermal amplification (LAMP) are used, they are more sensitive, accurate and suitable for species differentiation (Savan *et al*., 2004; Lan *et al*., 2008).

2.5.5 Control measures for of *Edwardsiella* infections

Edwardsiella infections can be treated by a number of antibiotics such as norfloxacin, ciprofloxacin, oxytetracycline, gentamicin, chloramphenicol, and aztreonam (Mohanty and Sahoo, 2007; Buller, 2014), which can be administered in

different ways such as systemic injection and in feeds. The overuse of antibiotics and other chemicals in fish farms needs to be monitored to avoid antimicrobial residue effect (Mohanty and Sahoo, 2007). The use of immunostimulants is a successful method of controlling most infectious diseases in fish (Woo and Bruno, 2011). There are commercially available vaccines for *E. ictaluri*. However, the commercially vaccines for *E. tarda* are still on trial and are not yet available (Park *et al*., 2012). The development and implementation of biosecurity programs in the fish farm is essential in reducing the risks of disease transmission and potential losses due to the disease outbreak (Mohanty and Sahoo, 2007; Palić *et al*., 2015).

2.5.6 Status of *Edwardsiella* infections

Edwardsiella infections, especially *E. tarda,* have been reported in tropical and subtropical areas of Africa, America, Asia, Europe and Australia. Its prevalence in ponds is seldom above 5% though it can reach 50% when fish are in confined tanks (Mohanty and Sahoo, 2007). In Africa, *E. tarda* has been reported in Chad, Ethiopia, Madagascar and East Africa (Woo and Bruno, 2011; Akoll and Mwanja, 2012; Habtamu and Kebede, 2016). *Edwardsiella ictaluri* has only been reported in Asia, Europe and America. *Edwardsiella ictaluri* is the most prevalent disease and mortality causing bacterium in the channel catfish (*Ictalurus punctatus*) in temperate countries (Declercq *et al*., 2013). The growth of *E. ictaluri* is limited to a certain temperature range, a factor that precludes it from being a pathogen for warm-blooded animals (Buller, 2014).

2.5.7 Economic and public health importance of *Edwardsiella* infections

Edwardsiella infections cause mass mortalities in infected fish leading to a serious economic loss in aquaculture (Park *et al*., 2012; Buller, 2014). In addition, *E. tarda* is a zoonotic pathogen and humans get the infection from either handling or eating infected fish (Wyatt *et al*., 1979; Alcaide *et al*., 2006; Park *et al*., 2012). The infection in human beings may manifest through gastrointestinal symptoms such as diarrhoea (Clarridge *et al*., 1980). Extra intestinal symptoms are also reported depending on the route of exposure such as endocarditis, empyema, hepatobiliary infections, peritonitis, intra-abdominal abscesses, osteomyelitis, wounds, and meningitis (Mohanty and Sahoo, 2007; Slaven *et al*., 2001).

CHAPTER THREE

3.0 Materials and methods

3.1 Study Area

The study was conducted in three districts of Morogoro Region, namely Morogoro Urban, Mvomero and Morogoro Rural (Fig.1) from November 2016 to April 2017. Three out of six districts were selected because they had more active fish farmers and big number of *O. niloticus* and *C. gariepinus* farms owned by the Government, institutions, and individual farmers. Laboratory work was performed in the Department of Veterinary Medicine and Public Health, in the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture (SUA).

3.2 Study design

This study adopted a cross-sectional study design whereby the selected fish farms were visited once and sampled followed by administering of questionnaires to fish farmers. From the selected farms, fish samples were randomly selected in fishing net and water quality parameters (temperature, dissolved oxygen, and pH) were assessed and further laboratory analyses were performed in the Department of Veterinary Medicine and Public Health laboratory, SUA.

3.3 Sample size estimation and sampling methods

Sample size was calculated using the following formula: $n = Z^2 P (1-P)/L^2$ (Naing *et al.,* 2006); where *n* is the required sample size, *Z* is the confidence interval = (1.96), *P* is the prevalence of the disease (for unknown prevalence as the case in this study, 50% is recommended), *L* is the expected error = 0.05. Sample size was

$=1.96\times0.5(1-0.5)/ (0.005)^2$ = 387 fish. A total of 400 live fish were supposed to be sampled but only 270 were sampled because most of the fish farms had already harvested during the study time.

3.3.1 Sampling and sample analyses

The Regional Fisheries Officer helped to identify the three districts with more fish farmers based on the available regional data. From each district the Fisheries Officers assisted in identifying villages with at least two farmers. With the help of local administration in each village, potential fish farmers were identified and visited. Farmers who were willing to participate in the study signed the consent form after explaining the aim of the study (Appendix 4).

3.3.2 Management practices

Questionnaires were administered to each fish farmer whose pond(s) were sampled to gather information about fish feeding, source of water, fries, feeds and feeding. Also the use of manure, water exchange programmes and history of mortalities (Appendix 2). During interviews, the questions were translated into Kiswahili. The biodata sheet was also used to record other observations (Appendix 3)

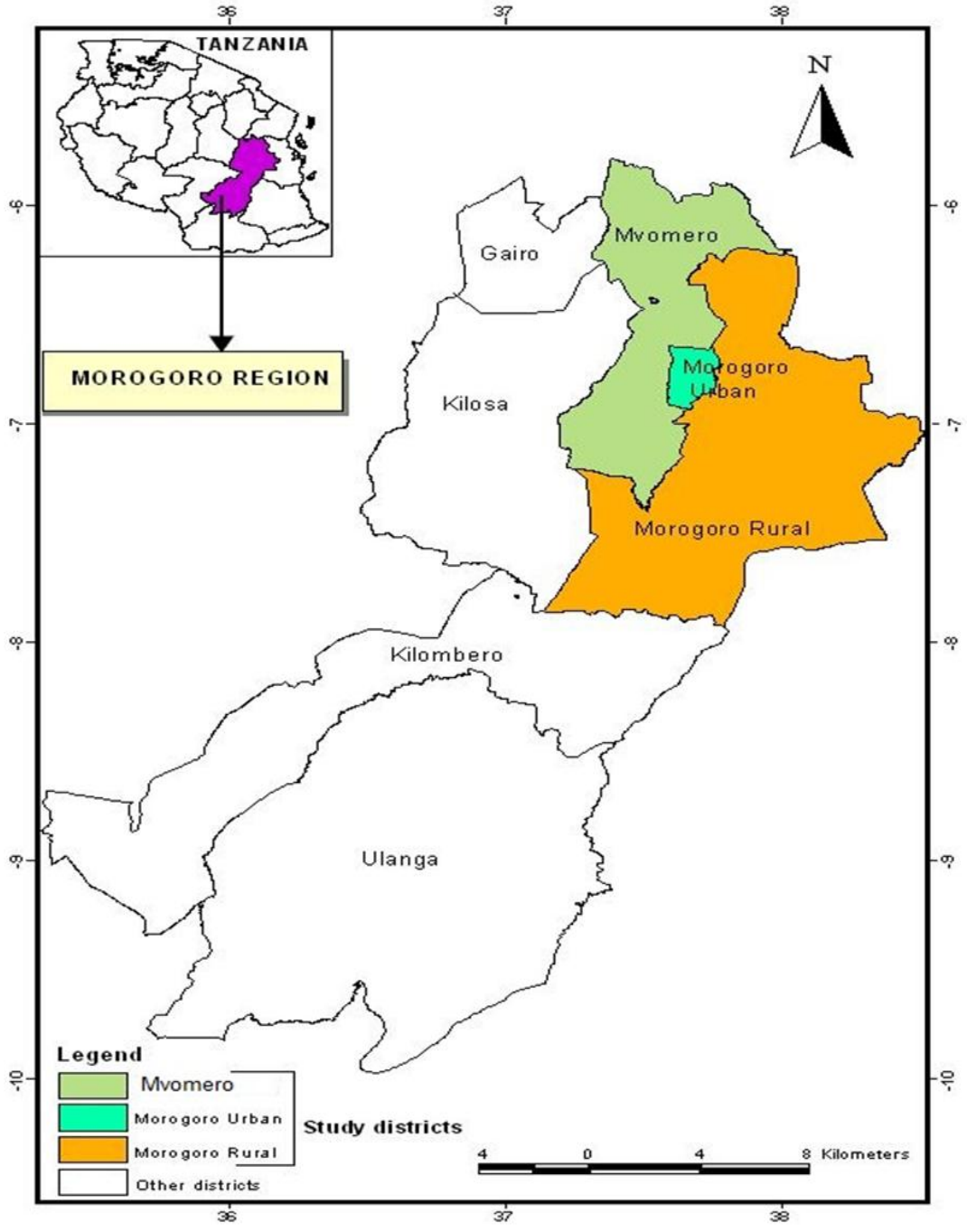

Figure 1: Map showing Morogoro region with its districts (Insert is the map of Tanzania which shows the location of Morogoro Region)

3.3.3 Water quality parameters assessment

From each pond, physico-chemical water quality parameters were recorded on-site, at two points, at the water inlet and outlet using portable dissolved oxygen meter (Milwaukee, USA) before sampling the fish. The assessed physico-chemical parameters were water pH, temperature and dissolved oxygen level. Ninety (90) millilitres of water from each pond were taken in sterile tubes for isolation *Edwardsiella* species.

3.3.4 Fish sampling

A total of 15 live fish were sampled randomly from each pond after fishing. In the pond, fish were caught gently using a gill net (Fig.4). The sampled fish were placed in a large clean container filled with pond water and immediately transported to SUA for further analyses. In the laboratory, each fish was clinically examined, weighed using digital weighing balance, and their lengths (girth, standard and total length) were determined using a tape measure. To minimize stress before collecting blood, fish heads were covered by wet towels then 2 ml blood samples were collected from each fish with a standard length greater than 8 cm via the caudal vertebral blood vessels using 5 ml sterile syringe with 20 gauges (Campbell, 2004). Then the blood was immediately transferred to the vacutainer tubes coated with EDTA and stored in the refrigeration at temperatures of $4^{\circ}C$ for not more than 2 hours before being analysed. The fish were later sacrificed by pithing (Mdegela *et al*., 2006) in order to collect samples, the fish were dissected aseptically, and swabs from spleen, kidney and liver were collected from each fish and stored in the nutrient broth for 12 hours under room temperature before bacteriological analysis.

3.4 Laboratory sample analysis

3.4.1 Culture and bacterial isolation

Swabs and pond water samples were cultured onto Tryptic soy agar (TSA) plates and incubated at 30 ˚C from 24 to 48 hours (Wang *et al*., 2011). Colony morphology was studied, small diameter, convex and transparent colonies were picked and sub cultured onto selective media, Salmonella-Shigella agar (SSA) and incubated at 30˚C from 24 to 48 hours. On SSA either colourless or with black spot colonies were picked and sub cultured in the same media to get pure colony for biochemical tests (Buller, 2014).

3.4.2 Gram staining and biochemical identification

Gram staining and biochemical tests such as catalase test, indole and hydrogen sulphide production, fermentation of glucose, methyl red, Simmons citrate test, Voges-Proskauer test, urease test, lysine decarboxylase were performed using conventional tests (Hemrej *et al*., 2013), for the identification of *Edwardsiella* species. Colony morphology and biochemical results were used as presumptive identification. The isolates which were Gram negative small rods, positive for indole, catalase, hydrogen sulphide production, lysine decarboxylase and negative for Voges-proskauer (VP), Simmon (citrate) and urease test were considered positive for *E. tarda*, while those negative for indole, urease, and hydrogen sulphide production and positive for VP test, methyl red and catalase test were considered positive for *E. ictaluri*. The identified isolates were taken for molecular analysis.

3.4.3 Molecular detection of *Edwardsiella* isolates

Since biochemical identification bases on Gram staining and biochemical test were for presumption identification and conformation was based on PCR through conventional PCR through detection of *gyr*B, inter-ribosomal spacer and an intervening sequence in the 23S rRNA gene for *E. tarda* and *E. ictaluri,* respectively.

3.4.3.1 DNA extraction

Isolates were cultured in the nutrient agar at 37 ˚C for 24 hours, a colony, which was cultured overnight, was added into the 30 µl sterile DNase-free distilled water; the mixture was boiled at 100 ˚C for 10 minutes using water bath and centrifuged at 1800 rpm for 5 minutes to sediment the cell debris and isolate genomic DNA. The DNA-containing supernatants were transferred to fresh Eppendorf tubes. For each reaction, 5 µl of supernatant was used as DNA template.

3.4.3.2 DNA amplification and analysis of PCR product

The DNA amplification was targeting divergent region of the partial *gyr*B gene for *E. tarda* using specific primers (Lan *et al*., 2008) and for *E. ictaluri,* two features of the ribosomal DNA gene clusters were targeted. The first feature was the presence of two ribosomal gene clusters located in tandem to one another, the inter-ribosomal spacer (IRS); and the second feature was the presence of an intervening sequence (IVS) in the 23S rRNA gene (Williams and Lawrence, 2010). The primer sets used in this study are depicted in Table 2 (Lan *et al*., 2008; Williams and Lawrence, 2010).

3.4.3.3 Primers preparation

Primers from the manufacturer were reconstituted by centrifuge at 8000 rpm to get stock solution of *gyr*B primers, 4175 µl were added to the 100 µl of primer and stored at -21˚C. In the IVR primers, 577.12 µl were added to the 100 µl of the primer. Then 10 µl of each prepared stock solution were taken to prepare working solution by the addition of 90 µl of distilled water and stored at -21˚C.

3.4.3.4 PCR mixture and PCR conditions

The Master mix for each targeted species consisted of 12.5 µl of premix, 1.2 µl of primers, 6.3 µl of distilled water and 5 µl of DNA samples were used in each extracted DNA. For the amplification of *gyr*B gene, the conditions included 30 cycles at 94˚C for 1 minute, 51.5 ˚C for 30 seconds, and 72 ˚C for 30 seconds, and an extra extension step of 72˚C for 10 minutes. The volumes (8 µl) of each PCR product were then subjected to electrophoresis in a 1% (w⁄ v) agarose gel while the amplification of IVR/IVS were conducted under the following conditions: 95 ˚C, 4 minutes; 30 cycles (95˚C, 30 seconds; 60˚C, 30 seconds; 72 ˚C, 2 minutes; and 72˚C, 10 minutes. The amplified products were separated on 0.7% agarose gel.

3.4.3.5 Gel electrophoresis and interpretation

The amplicons were separated on the respective agarose gels percent (1%), which was stained with ethidium bromide 0.005% (v/v). For every sample, 8 µl of the amplicon was charged into the respective wells of the agarose gel bathed in 1X TBE buffer made by 1:10 dilution of 10X TBE buffer. Three microliters of 100-1500 bp DNA ladder were mixed with 3 µl of loading dye and used as a size marker for the PCR products. The mixture was loaded into the agarose wells. The agarose gels were

electrophoresed at 110 volts for 60 minutes and visualized under UV illumination. The presence or absence of the appropriate sized band was recorded.

Table 2: Primer sets used in amplification of targeted bacterial genes

Bacteria	Gene	Primer sequences	Expected size
E. tarda	*gyr*B	*gyr*BF1 (5'-GCATGGAGACCTTCAGCAAT-3')	415 bp
		*gyr*BR1 (5'-GCGGAGATTTTGCTCTTCTT-3')	
E. ictaluri	23S	IVS (5'- TTA AAG TCG AGT TGG CTT AGG G-3')	1300 bp
	rRNA	IRS (5'-TAC GCT TTC CTC AGT GAG TGT C -3')	

3.5 Haematological analysis of the fish blood

Fish Blood was analysed for cell counts, haemoglobin estimation, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and packed cell volume. The analysis was done using an automatic haematology analyser (MS4s, Germany). Blood parameters were determined according to the guidelines proposed by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The reference values used were according to Hrubec *et al*. (2000) and William *et al*. (2016).

3.6 Data Analysis

Data were entered and coded in the Microsoft excel (2013) and then transferred to Epi Info 7 statistical package for analysis. Descriptive statistics was used to analyse frequencies, means and percentages. Normally distributed data were reported in the mean and standard deviation while those which were not normally distributed were

reported in the median and range. The proportional dichotomous variables were computed and compared using Fischer' exact test because the number of positive was low (only four positive) at critical probability of $\alpha < 0.05$ to establish the prevalence of Edwardsiella organisms. Logistic regressions were carried out to determine the association of the infection and the risk factors such as water quality parameters, host factors (size, sex and species) and management practices. The P value was used for comparison and odds ratio was also calculated to show the likelihood of infection.

CHAPTER FOUR

4.0 RESULTS

4.1 Management practices

Questionnaires were administered to 24 fish farmers and the results are presented in Table 5. The responses were complemented by direct observations. In the study area, farmers were practicing small scale fish farming, *O. niloticus* were the most commonly farmed fish in all the districts. Few farmers in the Urban were practicing polyculture by mixing *O. niloticus* and *C. gariepinus*. Earthen ponds were commonly used in Morogoro Rural and Mvomero but were poorly constructed making the exchange of water difficult. On the other hand, concrete ponds which were found in urban areas were either circular or rectangular in shape with clear inlet and outlet of water. In some areas of Morogoro Rural and Urban, draining of pond was not practiced in most fish farms due to water scarcity.

Farmers used organic animal manure to fertilize their ponds. The type of manure used to fertilize fish ponds depended on the species of animals kept by the farmer thus either chicken or cattle manure is used. However, the amount of manure used was not calculated to correspond to the pond size as a result, some farmers over fertilized their ponds. Most of the farmers used supplementary feeds but only 13% farmers used the formulated feed while the rest used maize bran, greens and table leftovers as feeds.

Most of the farms used streams as a source of water but disinfection of incoming water was not practiced in all the farms visited. Pests (birds, reptiles and rats), vehicles and people accessibility to ponds were not controlled. In Mvomero and Morogoro Rural, farmers had their associations which allowed the sharing of some materials such as fishing nets and fingerings.

Farmers with concrete ponds harvested their fish (Nile tilapia) after six months while all farmed catfish were harvested after six to nine months. The rest of tilapia farms were doing partial harvesting by fishing only large fish and leaving the small fish to grow.

Health abnormalities which were reported in the fish farms included sudden fish death, cotton like lesions on the fish skin, gasping, wounds, uncoordinated swimming and stunted growth. In addition and mostly in urban areas, about 36% of fish farms used different types of antibiotics such as oxytetracycline and Sulphur derivatives to manage reported diseases.

4.2 Physico-chemical water parameters

Water parameters were measured in all fish ponds and the results are as shown in Table 6. The amount of dissolved oxygen in Morogoro Rural was significantly lower ($p< 0.006$) than that of other districts and, the temperature in the District was ($31.2^{\circ}C$) higher than that in other districts although it was not statistically significant.

4.3 General description of sampled fish

A total of 270 fish were collected, clinically examined and sampled for different analyses. The distribution of the sampled fish in the districts and their average sizes is summarized in Tables 3 and 4. About 99% of *O. niloticus* were clinically normal while 1% had pale gills, abnormal eyes and abscess like swelling in their intestines (Fig. 3). On the other hand, 6% of *C. gariepinus* had different clinical signs such as abnormal swimming, swollen abdomen, emaciated, and scoliosis (Fig. 2).

Figure 2: *Clarias gariepinus* with different abnormalities (arrowed); - A: abdominal swelling B: scoliosis on backbone

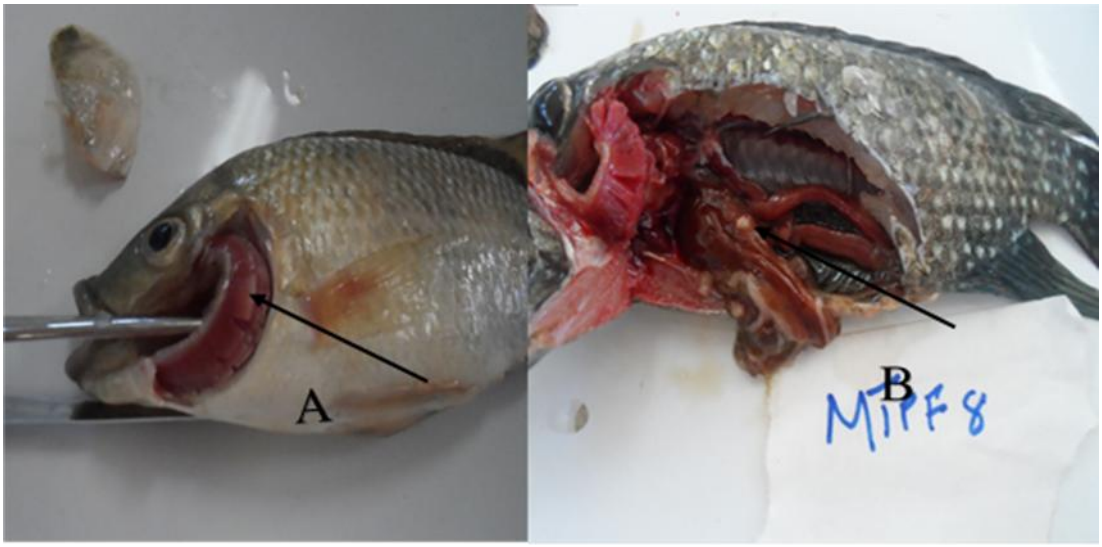

Figure 3: *Oreochromis niloticus* with different lesions (arrowed): A: pale gills and B: whitish nodule-like on the intestines

Figure 4: Show different sampled ponds; - A: poor managed earthen pond. B: over fertilized water with dark green colour on concrete pond.

Table 3: Frequency distribution of sampled fish Morogoro region

District	Number of pond	Type of fish		Sex		Total number of fish
				Male	female	
Morogoro urban	10	*O. niloticus*	80	50	30	110
		C. gariepinus	*30*	20	10	
Mvomero	10	*O. niloticus*	80	45	25	110
		C. gariepinus	30	10	20	
Morogoro rural	6	*O. niloticus*	40	23	18	50
		C. gariepinus	10	6	4	

Table 4: Weight and standard length of sampled fish in mean and standard deviation

Location	Species	Weight (g)	Standard length (cm)
Urban	*O. niloticus*	77.58±41.6	13.2±2.7
	C. gariepinus	55.24±97.8	13.9±10.1
Mvomero	*O. niloticus*	79.13±36.27	13.64±2.19
	C. gariepinus	315±270	25±8.8
Morogoro Rural	*O. niloticus*	44.94±39.2	10.7±2.72
	C. gariepinus	53.4±17.2	13.08±7.17

Table 5: Farmer's response on management practices

Parameter	Response	Frequency (n=24)
Farms that used antibiotic as treatment/Prophylactic	Yes	25%
Farms that do cleaning before stocking fish	Yes	54%
Farms that reported any health problem	Yes	38%
Farms which had water exchange program at least once in the month	Yes	50%
Farms that practice polyculture	Yes	29%
Farms that use well formulated feeds	Yes	33%
Farmers that fertilized their ponds using animal manure	Yes	58%
Maintaining the proper stocking density	Yes	42%
Types of pond that farm has	Earthen	67%
	Concrete	33%
Source of water in farm	Stream/well	67%
	Tape	33%

Table 6: Physico-chemical water parameter values in mean and recommended range in brackets

Location	Parameter		
	pH (6-8)	**Dissolve oxygen (mg/l) (4-10)**	**Water temperature (°C) (23-35)**
Urban	9.08±1.044	9.49±4.78	26.2±1.78
Morogoro Rural	7.6±0.93	3.92±0.97	31.02±3.93
Mvomero	8.64±1.51	9.03±3.93	27.7±4

4.4 Hematological analysis

Blood samples from 90 *O. niloticus* and 40 *C. gariepinus* were analysed for haematological parameters. The ranges of the remaining values which were obtained from 95% media are summarized in Table 7.

Table 7: Hematological values for sampled tilapia and catfish

parameter	*O. niloticus*			*C. gariepinus*		
	Range	**Median**	**Reference interval**	**Range**	**Median**	**Reference Value**
Leucocyte (m/mm^3)	1.3-7.15	7.15	21-154.7	1.52-50.51	16.44	3.6
Erythrocytes (M/mm^3)	0.51-1.62	0.78	1.91-2.83	0.5-1.8	1.21	1.85
Mcv (fl)	113.5-151.7	140.55	115-183	99.4-144.3	120.2	76
Pvc (%)	5.7-15.9	10.9	27-37	5-23.6	15.4	14
Mch (pg.)	23.5-56.8	44.8	No reports	28.8-45.5	36.55	25
Mchc (g/dl)	20.3-65.1	33	22-29	18-37	29.9	33
Hemoglobin (g/dl)	0.8-7.1	3.6	7-9.8	0.9-6.3	4.65	4
Thrombocyte (m/mm^3)	5-70	23	25-85	15-239	100.5	No reports

4.5 Isolation of Edwardsiella species

A total of 270 fish were sampled from 24 ponds from different districts. A swab of kidney, liver, spleen were collected from each fish making a total number of 810 organ swabs (Table 3). All swabs and pond water (24 ponds) were cultured. Onto TSA, small, convex and transparent colonies were picked and sub cultured on SSA. Colonies' size and characteristics were observed. Based on the colonies morphology phenotypic identification, isolates from 12 different fish showed characteristics of *E. tarda* whereas isolates of two other fish had similar characteristic with *E. ictaluri.* Therefore, the presumptive result was that a total of 14 fish had *Edwardsiella*

infections. Out of 14 fish, *O. niloticus* were four and *C. gariepinus* were ten. The results of organs and pond water are presented in Table 8.

Table 8: Frequency of the isolated *Edwardsiella* according site based on presumptive results

Sample site	**Species**	***Edwardsiella* positive (%)**
Kidney	*O. niloticus*	(n=200) 1.5%
	C. gariepinus	(n=70) 7%
Liver	*O. niloticus*	(n=100) 1%
	C. gariepinus	(n=70) 4%
Spleen	*O. niloticus*	(n=200) 0
	C. gariepinus	(n=70) 3%
Pond water		(n=24) 0

4.6 Molecular characterization of Edwardsiella isolates

All isolates from 14 positive fish were amplified by PCR using two different sets of primer specific for *E. tarda* and *E. ictaluri*, respectively, for confirmation. The PCR results were as follows, four out of 14 samples produced strong bands with the expected size based on amplification of *gyr*B gene hence they were confirmed to be positive for *E. tarda* (Fig. 5) while there was no amplicon on the IVS / IRS PCR assay meant that *E. ictaluri* was not isolated.

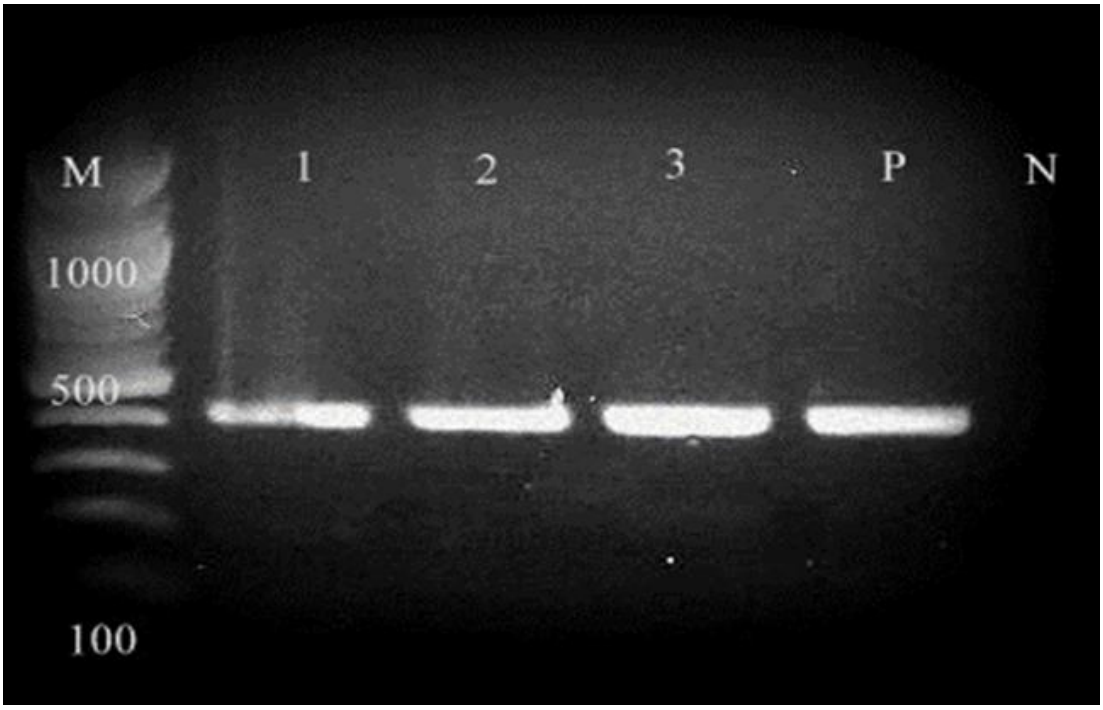

Figure 5: Agarose gel showing 415 bp PCR amplified products for detection of *E. tarda*. Lane M = 100 bp DNA Ladder; Lane 1, 2, 3 are samples, P = Positive control and N =Negative control

In (Fig. 5) lane 1, 2, and 3 are among the four positive isolated and positive control have bands at 415-bp marker which is the expected base pair for *E. tarda*. Four positive isolates each isolated from individual farmed fish in different districts; three *C. gariepinus* and one *O. niloticus* from Morogoro Urban and one *C. gariepinus* from Morogoro Rural. Although the bacteria were more prevalent in Morogoro Urban than in Morogoro Rural, the difference between the two districts was not statically significant ($p<0.827$). Therefore, based on confirmatory diagnosis based on PCR, the overall prevalence of *E. tarda* was 1.48% (n = 270) in this study.

4.7 Risk factors associated with *E. tarda* infections

Several factors were assessed using logistic regression and the results are summarized in Table 9. It was only one factor, where fish species showed to have a

relationship with the occurrence of *E. tarda* ($p<0.05$). Since it was the only value, multivalent analysis was not carried out.

Table 9: Univalent analysis of several predictors for occurrences of bacteria underlined are values when P≤ 0.05

Parameters	**Odds Ratio**	**95%**	**C. I**	**Coefficient**	**S. E**	**P-Value**
Age of fish	0.7881	0.0669	9.2885	-0.2381	1.2587	0.8499
Fish species	0.0814	0.0079	0.839	-2.5081	1.1901	<u>0.0351</u>
Sex of fish	2.9466	0.2882	30.123	1.0806	1.1861	0.3622
Size of the fish	1.2307	0.9211	1.6444	0.2076	0.1479	0.1603
Weight of the fish	0.9609	0.9063	1.0188	-0.0399	0.0298	0.1814
Water temperature	0.9788	0.6954	1.3776	-0.0214	0.1744	0.9021
antibiotic uses	4	0.2502	63.9497	1.3863	1.4142	0.327
Water source	2.4646	0.1382	43.9627	0.902	1.4701	0.5395

Legend: C.I= Confidence Interval, S.E=Standard Error

CHAPTER FIVE

5.0 Discussion

The questionnaire results and field observations have shown that farmers practiced both extensive and semi-intensive fish farming system. Despite low productivity of these systems, farmers adopted them due to low-input costs. Similar observation was made in previous studies in the country (Shah *et al*., 2012; Mathew *et al*., 2014; Limbu *et al*., 2016). It was found that fish were not regularly supplemented and when this was done, it was done using unbalanced feeds. Unavailability and high cost of commercial feeds have resulted to on-farm made and food leftover to be the commonly used supplementary feeds. This situation was also observed by Kaliba *et al*. (2006), Mathew *et al*. (2014) and Limbu *et al*. (2016) in their studies conducted in Tanzania. Poor quality of supplemented feed might partly contributed to low fish growth rate as also reported by Ashley (2006). Partial harvesting practiced by some farmers in Mvomero and Morogoro rural areas may lead to overcrowding of fish in the ponds, taking into account the early sexual maturity and prolific nature of *O. niloticus* which may also have contributed to the observed stunted growth.

Good farm management sustains the health of fish by reducing the risk of the introduction of pathogens and its spread from the farm (Muir, 1994). However, 90% the visited farms were not practicing biosecurity principles hence pathogen introduction and its spread were likely to occur. Unlike in the studies conducted in USA and Canada (Delabbio, 2003) which observed that over 60% of farms were practicing biosecurity, disinfection/treatment of incoming water, and controlled

access of personnel were not observed, live fish and fishing nets were commonly shared between farms risking the spread of the disease once one of the ponds gets the infection. All these factors pose risks of transmission of the disease between farms as suggested in other studies (Delabbio, 2003; Oidtmann *et al*., 2014; Bhujel, 2014; Cunningham *et al*., 2014). Water scarcity in some areas of Morogoro was also reported in previous studies by Mathew *et al*. (2014), making regular exchange of water impractical. With time, this leads to the deterioration of the quality of water, which in turn increases organic matter load in the pond hence causing stress to farmed fish and subjecting them to opportunistic infection.

In fish farming, water quality plays a crucial role in the balance between the host and the pathogen. The physical-chemical water parameters in the pond are in a dynamic state, and in this study, they were only measured once during sample collection; the variation with different times of the day could have missed. Several studies (Alcaide *et al*., 2006; Park *et al*., 2012) have confirmed the existence of *E. tarda* in aquatic environment as opportunistic pathogen. This means the pathogen and host certainly interact with each other. *Edwardsiella tarda* infections can be exacerbated by several environmental conditions (Zheng *et al*., 2004). Temperature is one of the several factors, affecting both pond dynamics and fish growth (sultan *et al*., 2016). The observed water temperature ranges (Table 6) in the studied districts were within the acceptable range for farming tropical fish (Bhatnagar and Devi, 2013). Meanwhile the same temperature range is also sufficient for proliferation of most pathogenic bacteria (Robert, 2001; Zheng *et al*., 2004; Cunningham *et al*., 2014; Noga, 2010).

The amount of dissolved oxygen is another crucial environmental factor for fish farming. Desirable concentration of dissolved oxygen in the water for most fish is 5 to 10 mg per litres (Sultan *et al*., 2016). Therefore, deviation to that range affects fish survival in that water. The abundance of phytoplankton and water temperature affects the availability of dissolved oxygen in the water (Nonga, 2017). In the present study, the amount of dissolved oxygen measured in Morogoro rural was statistically lower than that in other two districts and even lower than the recommended range for optimal fish production (Table 6). The low amount of dissolved oxygen observed in Morogoro rural might have been influenced by higher water temperature recorded in that District (Table 6). Despite the lower dissolved oxygen levels in Morogoro District, the prevalence of infection was low. Tolerability of *O. niloticus* to wide environmental conditions and fluctuation of these parameters within 24 hours might be the reason for this low prevalence observed. Generally, the physico-chemical water parameters in this study had no significant relationship with the prevalence of *E. tarda which was* established in the studied area.

The prevalence of 1.48% Edwardsiella infection was established by this study in farmed fish in Morogoro and only one species of genus *Edwardsiella* was detected, *E. tarda*. The infection was detected in three of the twenty four sampled fish farms. When the infection was compared across districts statistically unjustifiable differences were found across the districts. However, more infections were found in Morogoro Urban than in other areas. This can be partly explained by the fact that fish farming is not the main activity of most farmers in the urban areas, and so they put little efforts in pond management. Similar findings are reported by Mathew *et al*.

(2014), who found that urban fish farms had more parasites than was the case with fish farms in the rural areas of Morogoro, and this was caused by poor management of the farmer.

The results of the prevalence observed in current study is in agreement in the previous study conducted in other countries by Mohanty and Sahoo (2007) which fall in a range of from 1 to 50 % . On the other hand, the prevalence which was established in the present study is lower than that reported in India (13.2 %), China (29.4%) and 6.6% in Brazil (Moratoria *et al*., 2000; Lan *et al*., 2008; Kumar *et al*., 2016). The low prevalence of *E. tarda* observed in this study is probably due to semi-intensive fish farming system practiced, as opposed to intensive system practiced in other areas. In the extensive and semi-intensive systems, deterioration of water quality parameters is minimal and the stocking density is low (two to three fish per square meter); hence, there is less stress magnitude (Mohanty and Sahoo, 2007).

Edwardsiella tarda infections in the fish affect blood cell parameters were such that red blood cells and their indices decreased while white blood cells increased (Benli and Yildiz, 2004; Palmeiro *et al*., 2007). In the present study due to low prevalence of infected fish, it was difficult to relate statistically the influence of *E. tarda* infections on the obtained haematological parameters; instead the results were compared with other reported haematological studies of fish elsewhere. Blood cell values, which were observed, were slightly different compared with that found in experimental studies by Benli and Yildiz (2004), Hrubec *et al*., (2000) on *O. niloticus* and Fargbenro *et al*., (2013) on *C. gariepinus*. In their experimental studies

which involved O. *niloticus* (Benli and Yildiz 2004; Oidtmann *et al*., 2013) and its hybrid (Hrubec *et al*., 2000), showed that the red blood cell parameters were slightly higher than that shown in the findings of this study. These differences may be influenced by the age of fish used, as well as nutritional and environment factors and water temperatures all of which were different from those recorded in the present study.

As for risk factors, this study considered such factors as management practices, age, size, sex, species of fish, and water quality parameters as the risk factors which could be associated with the isolated and prevalence of *E. tarda* infection. However, only farmed fish species have shown to be statistically associated with the isolated *E. tarda*. *Clarias gariepinus* has shown to be more predisposed (P=0.03) than did *O. niloticus*, which could probably be due to bottom feeding characteristics of *C. gariepinus.* This made fish to have direct contact with detritus and mud of the pond where *E. tarda* also inhabit. From other studies (Waoo and Bruno, 2011; Buller, 2014) both *O. niloticus* and *C. gariepinus* showed to be equally susceptible to *E. tarda* infection. It is difficult to ignore the involvement of other factors which were not statistically significant due to low disease prevalence which was established in this study.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In the present study *E. tarda* was isolated and its prevalence was estimated to be 1.48% in fish farmed in Morogoro, Tanzania. The confirmation of *E. tarda* in fish farmed in Morogoro alert the farmer to take preventive measures by following biosecurity principles to prevent possible disease outbreaks.

6.2 Recommendations

- Drug sensitivity test is recommended to be conducted to establish the right antibacterial drug to be used in case of disease outbreak.
- Also determination of virulent factors on isolated bacteria is important so as to understand its pathogenesis which will may help in protection measures such as vaccine development.

REFERENCES

Abdel-Hakim, N. F., Bakeer, M. N. and Soltan, M. A. (1999). Integrated fish culture with farm animals. *Annals of Agriculture Science* 37 (2): 1001–115.

Abraham, T. J., Prakash K. M., Adikesavalu, H. and Banerjee, S. (2015). Pathology of *Edwardsiella tarda* infection in African Catfish, *Clarias gariepinus* (Burchell 1822), Fingerlings. *Archives of Polish Fisheries* 23 (3): 141–148

Akoll, P. and Mwanja, W. (2012). Fish health status, research and management in East Africa: Past and present. *African Journal of Aquatic Science* 37 (2): 117–129.

Alcaide, E., Herraiz, S. and Esteve, C. (2006). Occurrence of *Edwardsiella tarda* in wild European eels *Anguilla anguilla* from Mediterranean, Spain. *Diseases of Aquatic Organisms* 73 (1): 77–81.

Almaw, G., Woldeyes, A., Adugna, M., Koran, T., Fen, A. and Wubete, A. (2004). Outbreak of *Aeromonas hydrophila* associated with the parasitic infection *Ichthyophthirius multifiliis* in pond of African catfish (*Clarias gariepinus*) Fingerlings at Sebeta. *Journal of Veterinary Internal Medicine* 18 (2): 109–116.

Ashley, P. J. (2007). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science* 104 (3): 199–235.

Austin, B. and Austin, D. A. (2012). Bacterial Fish Pathogen: Disease of Farmed Wild Fish. Fifth Edition. Springer Ltd, London. 643 pp.

Benli, A. K. and Yildiz, H. Y. (2004). Blood parameters in Nile Tilapia (*Oreochromis niloticus*) spontaneously infected with *Edwardsiella tarda*. *Aquaculture Research* 35 (14): 1388–1390.

Bhatnagar, A. and Devi, P. (2013). Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Sciences* 3 (6): 1980–2009.

Bhujel, R. C. (2014). A manual for Tilapia business Management. CABI publishing, Oxford. 193 pp.

Buller, N. B. (2014). Bacteria from Fish and Other Aquatic Animals. Second Edition*: A Practical Identification Manual,* CABI publishing. Oxford. 720 pp.

Camus, A. C., Durborow, R. M., Hemstreet, W. G., Thune, R. L. and Hawke, J. P. (1998). *Aeromonas* bacterial infections - motile Aeromonad septicemia. *Southern Regional Aquaculture Center* 478: 1–4.

Centers for Epidemiology and Animal Health (1995). Overview of Aquaculture in the United States. Fort Collins, Colo: US Dept Agriculture. 27 pp.

Chitmanat, C., Kitiwan T. and Wichan, N. (2005). The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* spp. in Tilapia (*Oreochromis niloticus*) fingerlings, Songklanakarin. *Journal of Science and Technology* 27: 359-364.

Clarridge, J. E., Musher, D. M., Fainstein, V. and Wallace, R. J. (1980). Extraintestinal human infection caused by *Edwardsiella tarda*. *Journal of Clinical Microbiology* 11(5): 511-514

Cunningham, F. L., Jack, S. W. and Hardin, D. (2014). Risk Factors Associated with Enteric Septicemia of Catfish on Mississippi Commercial Catfish Farms. *Journal of Aquatic Animal Health* 26 (2): 84–90.

Cunningham, F. L., Jack, S. W., Hardin, D. and Wills, R. W. (2012). Pond-level Risk Factors associated with Columnaris Disease on Mississippi commercial catfish Farms. *Journal of Aquatic Animal Health* 24 (3): 178–84.

Danba, E. P., Bichi, A. H., Ishaku, S., Ahmad, Buba, M. K., Bingari, U. M., Barau S. B. W. and Fidelis, U. F. (2014). Occurrence of pathogenic Bacteria associated with *Clarias gariepinus* in selected Fish farms of Kumbotso local Government area of Kano state, Nigeria. *Bajopas* 7 (2): 145–49.

Delabbio, J. L. (2003). Biosecurity in the Recirculation Sector of Finfish Aquaculture in the United States and Canada. Dissertation for award Doctor of Philosophy at State University, Blacksburg, Virginia, USA. pp. 1-77.

Fagbenro, O. A., Adepanesi, E. O. and Timoh, W .O. (2013). Haematological profile of blood of African catfish (*Clarias gariepinus,* Burchell, 1822) fed sunflower and sesame meal based diet. *Journal of fisheries and aquatic science* 8(1): 80-86.

FAO (2005). Aquaculture production, 2003. Year book of Fishery Statistics – Vol.96/2. Food and Agriculture organization of the United Nations, Rome. 207 pp.

FAO (2016). The State of World Fisheries and Aquaculture 2016. Contributing to Food Security and Nutrition for All. Rome. 200 pp.

FSDP (2010). Tanzanian fisheries sector development Programme, Ministry Of Livestock and Fisheries Development. United Republic Of Tanzania. 37pp.

Gormaz, J. G. (2014). Public health perspectives on aquaculture. *Current environmental health reports* 1: 227–238.

Hemraj, V., Diksha, S. and Avneet, G. (2013). A Review on Commonly used Biochemical test for Bacteria. *Innovare Journal of Life Science* 1 (1): 1–7.

Hirono, I. (2013). A random genome analysis of *Edwardsiella tarda* ETSJ54 : annotation of putative virulence- related genes. *Colombia* 17 (1): 69–83.

Hrubec, T. C., Cardinale, J. L. and Smith, S. A. (2000). Hematology and plasma chemistry reference intervals for cultured Tilapia (Oreochromis Hybrid). *Veterinary Clinical Pathology* 29 (1): 7–12.

Iregui, C. A., Guarin, M., Tibata V. M. and Ferguson, H. W. (2012). Novel brain lesions caused by *Edwardsiella tarda* in a Red tilapia (*Oreochromis spp*.). *Journal of Veterinary Diagnostic Investigation* 24 (2): 446–449.

Kaliba, A. R., Osewe, K. O., Senkondo, E. M., Mnembuka, B. V. and Quagrainie, K. K. (2006). Economic analysis of Nile tilapia (*Oreochromis niloticus*) production in Tanzania. *Journal of World Aquaculture Society* 37(4): 464–473.

Kaur, V. I. and Ansal, M. D. (2010). Efficacy of Vermicompost as Fish pond Manure – Effect on Water quality and Growth of *Cyprinuscarpio* (Linn.). *Bioresource Technology* 101: 6215-6218.

Keremah R. I. and Inko-Tariah M. B. (2013). Comparative study of Ectoparasite on Nile Tilapia (Oreochromis Niloticus) cultured under Integrated and Unintegrated Pond Systems. *African Journal of Biotechnology* 12 (19): 2711–2714.

Kolawole, O. M., Ajayi, K. T., Olayemi, A. B. and Okoh, A. I. (2011). Assessment of water quality in Asa River (Nigeria) and its indigenous *Clarias gariepinus Fish*. *International Journal of Environmental Research and Public Health* 8 (11): 4332–4352.

Kpogue, D. N. S., Mensah, G. A. and Fiogbe, E. D., (2013). A review of biology, ecology and prospect for aquaculture of *Parachanna obscura*. *Reviews in Fish Biology and Fisheries* 23 (1): 41–50.

Lan, J. X., Zhang, H., Wang, Y., Chen, J. and Han, Y. (2008). Isolation of an unusual strain of *Edwardsiella tarda* from Turbot and establish a PCR detection technique with the *gyr*B gene. *Journal of Applied Microbiology* 105 (3): 644–651.

Li, G. Y., Li, J., Xiao, P., Guo, Y. H. and Mo, Z. L. (2011). Detection of type III Secretion Gene as an indicator for Pathogenic *Edwardsiella tarda*. *Letters in Applied Microbiology* 52 (3): 213–219.

Li, K., Petersen, G., Barco, L., Hvidtfeldt, K., Liu, L. and Dalsgaard, A. (2017). *Salmonella weltevreden* in integrated and non-integrated Tilapia aquaculture systems in Guangdong, China. *Food Microbiology* 65: 19–24.

Mathew, C., Mdegela, R. H., Mwamengele, G. L. and Kassuku, A.A. (2014). Prevalence and Mean Intensity of Ectoparasite Infections in Pond reared Nile Tilapia (*Oreochromis niloticus*) in Morogoro, Tanzania. *Tanzania Veterinary Journal* 29 (1): 63–71.

Mdegela, R., Myburgh, J., Correia, D., Braathen, M., Ejobi, F., Botha, C., Sandvik, M. and Skaare J. U. (2006). Evaluation of the gill filament-based EROD assay in African sharp tooth catfish (*Clarias gariepinus*) as a monitoring tool for waterborne PAH-type contaminants. *Ecotoxicology* 15: 51–59.

Menanteau-Ledouble, S. and Lawrence, M. (2011). Use of Fluorescent immuno-chemistry for the detection of *Edwardsiella ictaluri* in Channel catfish (*Icterus punctatus*) samples. *Journal of Visualized Experiments* 51: 7–10.

Mgode, G. F., Mhamphi, G., Katakweba, A. S. and Thomas, M. (2014). Leptospira Infections in freshwater fish in Morogoro Tanzania: A Hidden Public Health Threat. Tanzania. *Journal of Health Research* 16 (2): 1–7.

Mohanty, B. R. and Sahoo, P. K. (2007). Edwardsiellosis in Fish: A brief review. *Journal of Biosciences* 32: 1–14.

Moremi, N., Manda, E., Falgenhauer, L., Imirzalioglu, C., Matee, M., Chakraborty, T. and Mshana, S. E. (2016). Predominance of CTX-M-15 among ESBL producers from environment and fish gut from the shores of Lake Victoria in Mwanza, Tanzania. *Frontier in Microbiology* 7: 1-11.

Muir, J. F. (1994). Water reuse systems in aquaculture. *INFOFISH International* 6: 40-46.

Mwita, C. and Nkwengulila, G. (2008). Determinants of the parasite community of clariid fishes from Lake Victoria, Tanzania. *Journal of Helminthology* 82: 7–16.

Naing, L., Winn, T. and Rusli, B. N. (2006). Practical Issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences* 1: 9–14.

NCCLS (2000). How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition. NCCLS document C28-A2 Pennsylvania. pp 25-27.

Noga, E. J. (2010). Fish disease: Diagnosis and treatment. Second Edition. Blackwell publishing, Iowa. 496 pp.

Nonga, H. E, Mdegela, R. H., Sandvik, M. Lie, E., Miles, C. O. and Skaare, J. U. (2017). Cyanobacteria and cyanobacterial toxins in the alkaline-saline Lakes Natron and Momela, Tanzania. *Tanzania Veterinary Journal* 32 (1): 108–116.

Oidtmann, B., Peeler, E., Lyngstad, T., Brun, E., Bang, B. and Stärk, K. D. C. (2013). Risk-based methods for fish and terrestrial animal disease surveillance. *Preventive Veterinary Medicine* 112: 13–26.

Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases* 35: 83–108.

Palić, D. A., Scarfe, D. and Walster, C. I. (2015). A Standardized approach for meeting national and international aquaculture biosecurity requirements for preventing, controlling and eradicating infectious diseases. *Journal of Applied Aquaculture* 27 (3): 185–219.

Palmeiro, B. S., Rosenthal, K. L., Lewbart, G. A. and Shofer, F. S. (2007). Plasma biochemical reference intervals for Koi. *Journal of the American Veterinary Medical Association* 230 (5): 708–712.

Park, B. S., Aoki, T. and Jung, S. S. (2012). Pathogenesis of and Strategies for Preventing *Edwardsiella tarda* Infection in Fish. *Veterinary Research* 43 (1): 67-78.

Park, S. B., Kwon, K., Cha, I. S., Jang, H. B., Nho, S. W., Fagutao, F. F., Kim, Y. K., Yu, J. E. and Jung, T. S. (2014). Development of a multiplex PCR assay to detect *Edwardsiella tarda, Streptococcus parauberis, and Streptococcus iniae* in Olive flounder (*Paralichthys olivaceus*). *Journal of Veterinary Science* 15: 163–166.

Pridgeon, J. (2012). Major bacterial diseases in aquaculture and their vaccine development. *CAB Reviews* 7 (48): 1–16.

Reichley, S. R. and Ware, C. (2015). Assays for the detection and quantification of *Edwardsiella tarda, Edwardsiella piscicida, and Edwardsiella piscicida–* like species in catfish tissues and pond water. *Journal of Veterinary* 27 (2): 130–139.

Robert, R. J. (Ed) (2001) Fish Pathology, Fourth edition. Black well publishing, London. 571 pp.

Savan, R., Igarashi, A., Matsuoka, S. and Sakai, M. (2004). Sensitive and rapid detection of Edwardsiellosis in fish by a loop-mediated isothermal amplification method. *Applied and Environmental Microbiology* 70 (1): 621–624.

Sechter, I., Shmilovitz, M., Altmann, G., Seligmann, R., Kretzer, B., Braunstein, I. and Gerichter, C. B. (1983). *Edwardsiella tarda* isolated in Israel between 1961 and 1980. *Journal of Clinical Microbiology* 17 (4): 669–671.

Shah, S., Duncan, Q. A., Colquhoun, J., Nikuli, H. L. and Sørum, H. (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environmental Science and Technology* 46 (16): 8672–8679.

Slaven, E. M., Lopez, F. A., Hart, S. M. and Sanders, C. V. (2001). Myonecrosis caused by *Edwardsiella tarda*: A case report and case series of extraintestinal *E. tarda* infections. Clinical infectious diseases. *An Official Publication of the Infectious Diseases Society of America* 32: 1430–1433.

Stacy, N. I., Arnold, J. E. and Boylan, S. (2014). Diagnostic heamatology of fish: Review and tips. In *Proceedings Association of Reptilian and Amphibian Veterinarians* 119–121.

Stock, I. and Wiedemann, B. (2001). Natural antibiotic susceptibilities of *Edwardsiella tarda, E. ictaluri, and E. hoshinae*. *Journal of Antimicrobial Agents and Chemotherapy* 45 (8): 2245–2255.

Subasinghe, R. P. (2005). Epidemiological approach to aquatic animal health management: Opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine* 67: 117–124.

TFAR (2014). Fisheries Annual Statistics Report, Ministry of Livestock and Fisheries Development, Fisheries Development Division. Tanzania. 20 pp.

Thrall, M.A., Weiser, G., Allison, R. W. and Campbell, T.W. (2012).Veterinary Hematology and Clinical Chemistry. Second Edition. Blackwell Ltd, Iowa. 750 pp.

Wang, Y. M., Wang, Q. Y., Xiao, J. F., Liu, Q., Wu, H. Z. and Zhang, Y. X. (2011). Genetic relationships of *Edwardsiella* strains isolated in China aquaculture revealed by Rep-PCR genomic fingerprinting and investigation of *Edwardsiella* virulence genes. *Journal of Applied Microbiology* 111 (6): 1337–1348.

Waoo, P. T. K. and Bruno, D. W. (Ed) (2011). Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections. Second Edition. CAB International, London. 941 pp.

Weiss, D. J. and Warddrop, K. J. (Ed) (2010). Shalm's Veterinary Hematology. Sixth Edition. Blackwell publishing, Iowa. 1176 pp.

Williams, M. L. and Lawrence, M. L. (2010). Verification of an *Edwardsiella ictaluri*-Specific Diagnostic PCR. *Letters in Applied Microbiology* 50 (2): 153–157.

Williams, M. L., Waldbieser, G. C., Dyer, D. W., Gillaspy, A. F. and Lawrence, M. L. (2008). Characterization of the *Rrn* operons in the channel catfish pathogen *Edwardsiella ictaluri*. *Journal of Applied Microbiology* 104 (6): 1790–1796.

APPENDICES

Appendix 1: Questionnaire for fish farmers

Date and time of interview: ____/____/2016/17 ____:____ a.m. /p.m.

Name of respondent:

_____

Farmer sex: _____ male □ female □

Location: _____

I. GENERAL FARM DESCRIPTION AND USE OF WATER

1. How many ponds do you have in farm? _____ Ponds

2. How big are the respective ponds (in m^2)? A: _____ B: _____ C: _____

3. What species of fish do you farm? (1 = Tilapia, 2 =catfish 3= both_____

4. When do you harvest your fish_____?

6. Where does the water come from in your Farm: (1 = Tape, 2 =stream 5= others ___

7. Does the water flow through other ponds, before entering the respective pond? (1 = paddy fields, 2 =ponds, 3 = no flow through previous fields/ponds, 4 = other(s);

8. Do you sometimes have to cope with water shortages? Yes □No □ If yes, in which months and how_____

9. Do you do water exchange to you pond _____ (1=yes, 2=No) If yes How often within a month_____

10. Are you able to drain the pond? _____ (1=yes, 2=No) If yes, does draining depend on other farming activities? _____

11. Do you dry-out/clean the pond before stocking? (1 = yes, 2 = no

II. STOCKING OF POND

1. When did you stock your pond(s)? _____

2. Where do you get your fingerlings_____

3. How do you estimate the quality of fingerlings_____?

III. FEED MANAGEMENT

1. Which ingredients do you use to make your fish feed? _____

2. How often do you feed per day? (1 = once, 2 = twice, 3 = seldom, 4 = never)

3. How much do you give them_____?

4. Do you use manure in your ponds? Yes □No □ If yes in which form____ (e.g. fresh

5. What kinds of manure do you usually use? _____ (1= cattle, 2 = chicken, 3= other(s) Please estimate the amount and frequency of manure application.___ (e.g. 3 twice a week)

7. How do you store your feed?

IV. FISH DISEASES

1. Have you ever had to cope with fish diseases in your farm? (1 = often, 2 = sometimes, 3= never)

2. Do you know the name of the disease/symptoms _____?

3. How many fish have died? _____ what was their size

4. Do you have any insight as to why your pond has been affected by disease?

5. Do you use antibiotic in disease treatment/prophylactic? Yes □No □ if yes which ones_____

Appendix 2: Water quality parameter record sheet forms

Name of the owner …… Pond number……location……

Pond	pH	Water temperature	DO
1			
2			
3			
4			
5			

Appendix 3: Sampled fish biodata sheet form

S/N	Species	sex	Weight	Length

Appendix 4: Client consent form

DEPARTMENT OF PUBLIC HEALTH AND VETERINARY MEDICINE

STUDY TITLE: EPIDEMIOLOGY OF *EDWARDSIELLA* INFECTION IN FARMED FISH IN MOROGORO, TANZANIA.

You are invited to participate in a research study aiming to contribute towards reduction of fish mortalities due to bacterial diseases. The study requires obtaining samples in fish ponds, after consulting the District fisheries officer we were directed to you. This study is being conducted by Mr. Emil Mkemwa, Masters Student in Health of Aquatic Animal Resources. Please read this form carefully and ask any questions you may have before agreeing to participate in this study.

Background and Purpose: Genus *Edwardsiella* comprising of four bacterial species (*E.tarda, E.ictaruli, E. piscicida* and *E .hoshinae*) which cause diseases in fish and E.tarda is zoonotic bacterium cause various syndromes to human being. Their infections lead to serious economic losses in the aquaculture worldwide. However, in Tanzania less is known on *Edwardsiella infection* in cultured species of fish. The aim of this study is to provide epidemiological information on *Edwardsiella tarda* in farmed fish in Morogoro. Finding will be communicated to ministry of livestock and fisheries for appropriate measures to be taken. There will be no treatment applied during the study.

Study Procedures: The samples will be collected in 30 days and it will involve, seining a maximum of 13 fish per pond, recording water quality parameters and request you to fill a questionnaire which may help us determine the risk factors for occurrence of bacterial diseases in you ponds. Fish sampled will be put into separate

covered containers and transported to the Microbiology laboratory at Sokoine University of Agriculture for analysis. Organs including kidney, spleen, and liver will be used for bacteriology and blood sample will be used for heamatology.

Risks of study participation: During sampling, pond floors may be destructed, and people involved in sampling may be pierced by fish spines. Stress caused by sampling may also cause mortalities on remaining stock.

Benefits of study participation: The benefits of study participation are direct payments of all sampled fish. People involved in seining during sampling will also be paid.

Study costs/compensation: We do not anticipate any risks to you participating in this study other than those encountered in day to day life. However if you experience unusual mortalities associated with sampling, the study will cover for the losses.

Confidentiality: All results will be confidential. Information about your animal (Fish and their environment) may be used in scientific presentations and/or publication. However, no personal or identifying information about you or your animal will be released. Your animals' records for the study may be reviewed by One Health Central and Eastern Africa donors and by departments at the Sokoine University of Agriculture with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

Voluntary Participation: Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University, local government or the community.If you decide to participate, you are free to withdraw at any time without affecting those relationships. Please do not hesitate to contact us if you have any questions or concerns about this study. You will be given a copy of this form to keep for your records.

Investigator; Emil Mkemwa

Phone number; 0717192521

Email: mkemwaemil@yahoo.com

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Client name……Signature ……Date……