

RISKS OF ENTEROPATHOGEN INFECTION IN HUMANS AND CATTLE  
ASSOCIATED WITH MANURE MANAGEMENT IN URBAN AND PERI-URBAN  
AREAS OF MOROGORO, TANZANIA

BY

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A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE  
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## EXTENDED ABSTRACT

Urban population growth has created an increase in demand for food including those of animal origin. In response to ample market, urban and peri-urban livestock farming has expanded both in the number of livestock and the number of households engaged in livestock keeping. As a result, human-animal interaction has increased and concurrent increase in manure production within the same land space has increased human-manure-environment contact. The present study was aimed at investigating cattle and manure management practices and associated risks of manure-borne enteropathogen infection to humans and animals and environmental contamination in urban and peri-urban settings of Morogoro, Tanzania.

One hundred and nineteen smallholder dairy cattle keeping households from urban and peri-urban areas of Morogoro municipal, Morogoro rural and Mvomero districts were randomly selected for the study. To each cattle keeping household, a non-cattle keeping neighbor from within a radius of 100m was selected and a pair formed a cluster. Administration of structured questionnaires to cattle keepers and non-cattle keepers, together with observations was used to collect information about cattle and manure management practices. Individual fecal samples were collected from all cattle present at the household registered for the study. Stool samples from individuals from cattle keeping households and non-cattle keeping neighbors as well as soil and water samples were collected for the purpose of isolating zoonotic *Salmonella* spp., non-sorbitol fermenting diarrheagenic *E. coli* and non-pathogenic commensal *E. coli* strains. In total,

there were 446 cattle fecal, 201 stool, 201 soil and 201 water samples for bacteria isolation.

Zoonotic *Salmonella* species were isolated on Salmonella-Shigella agar and characterized by biochemical and standard serological methods. Diarrheagenic *E. coli* were isolated from sorbitol MacConkey agar and characterized by conventional biochemical, serological and antimicrobial susceptibility tests and molecular methods such as PCR and DNA hybridization. The non-pathogenic *E. coli* isolated from MacConkey agar were screened for double resistance for ampicillin and tetracycline using Petri film Select *E. coli* count plate and later analysed for genetic relatedness by Pulsed Field Gel Electrophoresis (PFGE). Logistic regression was used to quantify the risks for transmission of *E. coli* between cattle, humans, water and soil based on PFGE results.

It was revealed that cattle from different herds were allowed to mix and there was indiscriminate defecation during grazing. Manure collection, conveyance and disposal resulted into direct human contact with manure and manure was disposed off within and around residential areas either as fresh or composted.

The prevalence of shiga toxin-producing *E. coli* (STEC) O157:H7 in cattle was 0.9% (95% CI: 0.29 – 2.15) while the prevalence of all STEC strains in cattle was 1.6% (95% CI: 0.69 – 3.08). The overall prevalence of diarrheagenic *E. coli* in cattle was 2.2% (95% CI: 0.99 – 3.67) and 0.5% (95% CI: 0.025 – 2.44) in water sources. This shows that cattle remain potential source of pathogenic *E. coli* to humans and

environment. Among the non-sorbitol fermenting *E. coli* isolates, one Extended Spectrum Beta Lactamase (ESBL)-producing isolate showed the Multilocus Sequence Typing (MLST) type ST131 that causes antimicrobial-resistant infections in humans.

Zoonotic Salmonella strains such as *Salmonella amager*, *S. kentucky* and *S. weltevreden* were isolated from humans and cattle. Overall, four samples out of 1046 (0.38%) were positive, while the prevalences of Salmonella in humans and cattle were 1% and 0.45% respectively. Isolation of *S. amager* in asymptomatic human subject shows that, the pathogen that was previously reported to cause gastroenteritis outbreak in humans, may have lost its virulence characteristics but still exists within the human population.

There was transmission of *E. coli* between cattle, humans, soil and water within and between clusters due to manure management. Out of 44 clusters from which ampicillin and tetracycline resistant *E. coli* were isolated, 16 clusters (36%) had at least one isolate that was 100% identical to another isolate but from another source within the same epidemiological unit. Cattle (OR=19.2, CI: 2.04-179.8) and manure (OR=0.4, CI: 0.17-0.89) management practices were the risk factors for *E. coli* transmission between cattle, humans, soil and water.

Cattle and manure management practices in urban and peri-urban areas of Morogoro put humans and animals at risk of infection with pathogens including *E. coli* O157:H7 and ESBL producing ST131 strains while contaminating the environment. Therefore, there is a need for formulation and enforcement of manure management guidelines that safeguard human, animals and the environment.

DECLARATION

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

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## DEDICATION

To my children Halima and Yahaya. You endured long periods of my absence while pursuing this study.

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## LIST OF ABBREVIATIONS

DAEC	Diffusely adherent <i>E. coli</i>
DEC	Diarrheagenic <i>E. coli</i>
DNA	Deoxyribonucleic acid
DTU	Technical University of Denmark
EAEC	Enteraggregative <i>E. coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ESBL	Extended Spectrum $\beta$ -Lactamase
ETEC	Enterotoxigenic <i>E. coli</i>
ExPEC	Extra-intestinal pathogenic <i>E. coli</i>
HUS	Hemolytic uremic syndrome
LRRD	Livestock Research for Rural Development
NIMR	National Institute for Medical Research
NSF	Non-sorbitol fermenter
PCR	Polymerase chain reaction
PFGE	Pulsed-field Gel Electrophoresis
STEC	Shiga toxin-producing <i>E. coli</i>
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
URT	United Republic of Tanzania
VTEC	Verocytotoxigenic <i>E. coli</i>

## CHAPTER ONE

### 1.0 INTRODUCTION

#### **1.1 Urban and peri-urban livestock farming**

In the last few decades, urban and peri-urban farming in developing countries has been progressively increasing. One reason for its emergence and expansion was a survival strategy of the urban dwellers to cope with reduced income and living standards (Briggs, 1991, Mlozi, 1996, Mlozi, 1997a, Mvena, 1999, DFID, 2002, Simon *et al.*, 2004). It was also a diversification tactic to spread livelihood risks in adverse situations (DFID, 2002). Some urban and peri-urban dwellers continued to keep livestock to maintain their rural cultural values (Mlozi, 1996, Mvena, 1999). The expansion of urban and peri-urban livestock farming is reflected in the increase in both the number of animals kept and the number of households engaged in keeping livestock. Urban areas of Morogoro, Tanzania, for example had a cattle population of 2,618 in 1996 (URT, 1997), which almost doubly increased to 4,170 in 2006 (URT, 2007). By 2008 the cattle population in Morogoro urban was 19,099 among them 4,425 were dairy cattle (URT, 2012a). In 1999, the city of Dar es Salaam had 14,000 cattle, which increased to 32,398 by the end of 2008 (URT, 2012b).

#### **1.2 Urban human population**

Continued increasing populations and associated high demands for animal protein has provided a boost to urban and peri-urban farming. Influx of people from rural areas is one among the primary causes of urban population growth in terms of either

migrants or commuters (Briggs, 1991, Simon *et al.*, 2004). For instance, Tanzania's annual population growth rate between 1988 and 2002 was 3% with the urban population size increasing from 18% in 1988 to 23% in 2002 (URT, 2002). A concurrent rise in food demand to feed the increasing urban population has created ample market for livestock products (Briggs, 1991, Simon *et al.*, 2004). Due to close vicinity of the peri-urban to urban market and high demand for food including animal products such as milk, meat and eggs in urban areas, peri-urban areas have become optimal places for livestock production (Sumberg, 1997).

### **1.3 Animal waste and pathogens**

Simultaneous to increase in animal population, the amount of animal waste and associated problems with its handling is on the rise. Despite intensification of livestock farming, animal waste disposal infrastructure has not been developed to match the increased animal waste volume. The distance separating animals from human has shrunk leading to close contact between animals, human and animal wastes. Consequently, poorly managed animal waste causes bad odor, provides favorable breeding grounds for flies and contaminate the environment (Mlozi, 1999). Moreover, mismanaged animal waste predisposes humans to pathogen infection (Crump *et al.*, 2002) which could otherwise be exclusive to livestock.

Examples of zoonotic pathogens that have veterinary and public health importance are the bacteria of the genera *Salmonella*, *Campylobacter* and *Escherichia* and protozoans such as *Cryptosporidium* and *Giardia* species (Smith *et al.*, 2004). These pathogens reside in the gastrointestinal tract of animals and feces are a shedding

vehicle (Heuvelink *et al.*, 2007); hence, they can contaminate the environment and/or infect humans during handling of animals and animal wastes (Crump *et al.*, 2002). Common disease syndromes caused by manure-borne pathogens include diarrhea, dysentery, hemorrhagic colitis and hemolytic uremic syndrome (Germani *et al.*, 1997, Smith *et al.*, 2004).

#### **1.4 Study area**

This study was conducted in urban and peri-urban areas of Morogoro region of Tanzania. Morogoro region typifies the relationship between dense urban populations as potential market with expanding urban and peri-urban livestock farming as source of food and income. Morogoro region is located between latitude 5° 58" and 10° 0" S and longitude 35° 25" and 35° 30" E. Ambient temperature ranges between 18°C and 30°C. The annual rainfall ranges between 600 mm – 1200 mm (URT, 1997). Three districts of Morogoro region namely Morogoro urban, Morogoro rural and Mvomero constituted the study area (Fig. 1). In total, nine wards, namely Kihonda, Bigwa, Kingolwira, Kilakala and Kichangani (Morogoro urban), Mkambarani and Mikese (Morogoro rural), Mzumbe and Mlali (Mvomero) were investigated.

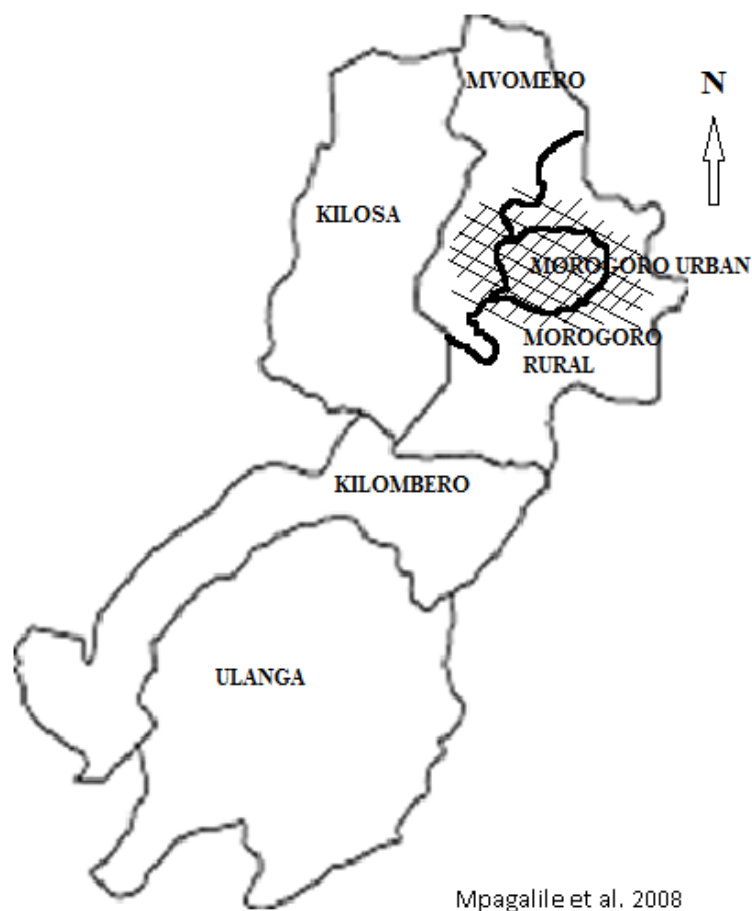


Figure 1: A map of Morogoro region showing the study area

### 1.5 Research Problem and Justification

Isolation of *Salmonella* species, *Campylobacter* species and *E. coli* O157:H7 from freshly voided animal feces (Heuvelink *et al.*, 2007) put human at risk of infection and environmental contamination. Cases of human gastroenteritis due to bacteria enteropathogens of animal origin have been widely reported (Kapperud *et al.*, 2003, Merritt and Herlihy, 2003, Hendriksen *et al.*, 2004, Smith *et al.*, 2004). Many studies have been conducted about urban and peri-urban farming in Tanzania and elsewhere and about changes in land use (Briggs, 1991, Bah *et al.*, 2003, Simon *et al.*, 2004), and some studies reporting on economic (Mlozi, 1996, Mlozi, 1997a) and



environmental effects of urban and peri-urban livestock farming (Mlozi, 1999). Yet no study has been carried out to assess the relationship between the expansion of urban and peri-urban livestock farming, animal waste management and health repercussion in animals and humans. Isolation of pathogenic fecal bacteria of animal origin in humans e.g. isolation of *E. coli* O157:H7 from diarrheagenic patients in Morogoro hospital by Raji *et al* (2008) together with increased contact between humans and animals in urban and peri-urban areas has led to speculation that animal feces could be the source of human infection. Therefore, this study aimed at assessing the risk of transmission of zoonotic fecal bacteria between animals, humans and the environment in urban and peri-urban livestock farming system in Tanzania and its relationship with animal waste management.

## **1.6 Study Objectives**

### *1.6.1 Main study objective*

To determine the risks of fecal bacteria transmission between cattle, humans and the environment in urban and peri-urban livestock farming areas in Morogoro, Tanzania.

### *1.6.2 Specific objectives*

- i. To assess current cattle and manure management practices and to identify risk factors for transmission of pathogens between cattle and humans in urban and peri-urban livestock farming areas in Morogoro, Tanzania.
- ii. To determine the occurrence of zoonotic *Salmonella* and pathogenic *E. coli* strains in cattle, humans and the external environment in urban and peri-urban livestock farming areas in Morogoro, Tanzania.

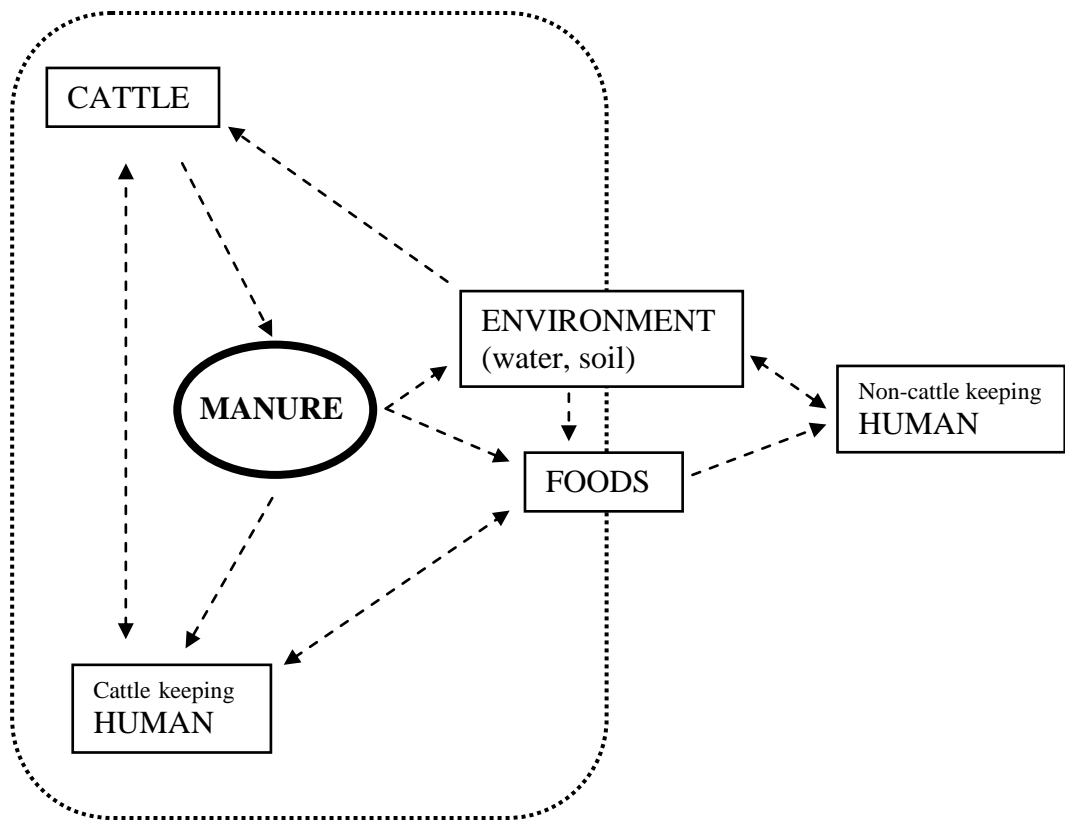
- iii. To identify the risks for transmission of zoonotic *Salmonella* strains and pathogenic *E. coli* between cattle, humans and the external environment in urban and peri-urban livestock farming areas in Morogoro, Tanzania.
- iv. To establish the genetic relatedness of *E. coli* isolates from animals, human and the external environment in urban and peri-urban livestock farming areas in Morogoro, Tanzania.

### **1.7 Study design**

The study involved smallholder dairy cattle keeping households and their non-cattle keeping neighbors in urban and peri-urban areas of Morogoro, Tanzania. The hypothesis was that transmission of enteropathogens occurred during manure collection, utilization and disposal between cattle, soil, water and humans (both cattle keepers and non-cattle keepers) as conceptualized in Figure 2. A sample of 119 households who owned 806 cattle was randomly selected from a sampling frame of 367 cattle keeping households. To each selected cattle keeping household, a non-cattle keeping neighbor was selected from within a radius of 100m. In a study to determine the prevalence of diarrheagenic *E. coli* each cattle keeping household was regarded as a cluster, while in a study to determine the transmission of *E. coli* isolates from cattle, humans and the environment a cluster was composed of a pair of cattle keeping household and a neighboring non-cattle keeping household. During the baseline survey, observations and semi-structured questionnaires were used to gather information about cattle and manure management practices from all cattle and non-cattle keepers who were selected for the study. However, the number of participants in other studies that involved sample collection varied according to requirements and

willingness of participants to avail samples. Stool samples were collected from one member of the household in addition to soil and water samples from each participating household. Individual cattle fecal samples were collected from each animal at the household. Isolation and characterization of *E. coli* and salmonella strains was done according to procedures summarized in figure 3.

Data from this study were analysed both descriptively and analytically. Descriptive statistics such as means, frequencies and cross-tabulation were used to summarize the data from preliminary survey while logistic regression analysis was used to identify risk factors for transmission of *E. coli* between cattle, humans, water and soil.

**Key**

- ..... Cattle keeping household boundary
- - - - -> Possible direction of pathogen transmission

Figure 2: Hypothetical transmission pathways of enteric bacteria in urban and peri-urban livestock farming systems in Tanzania

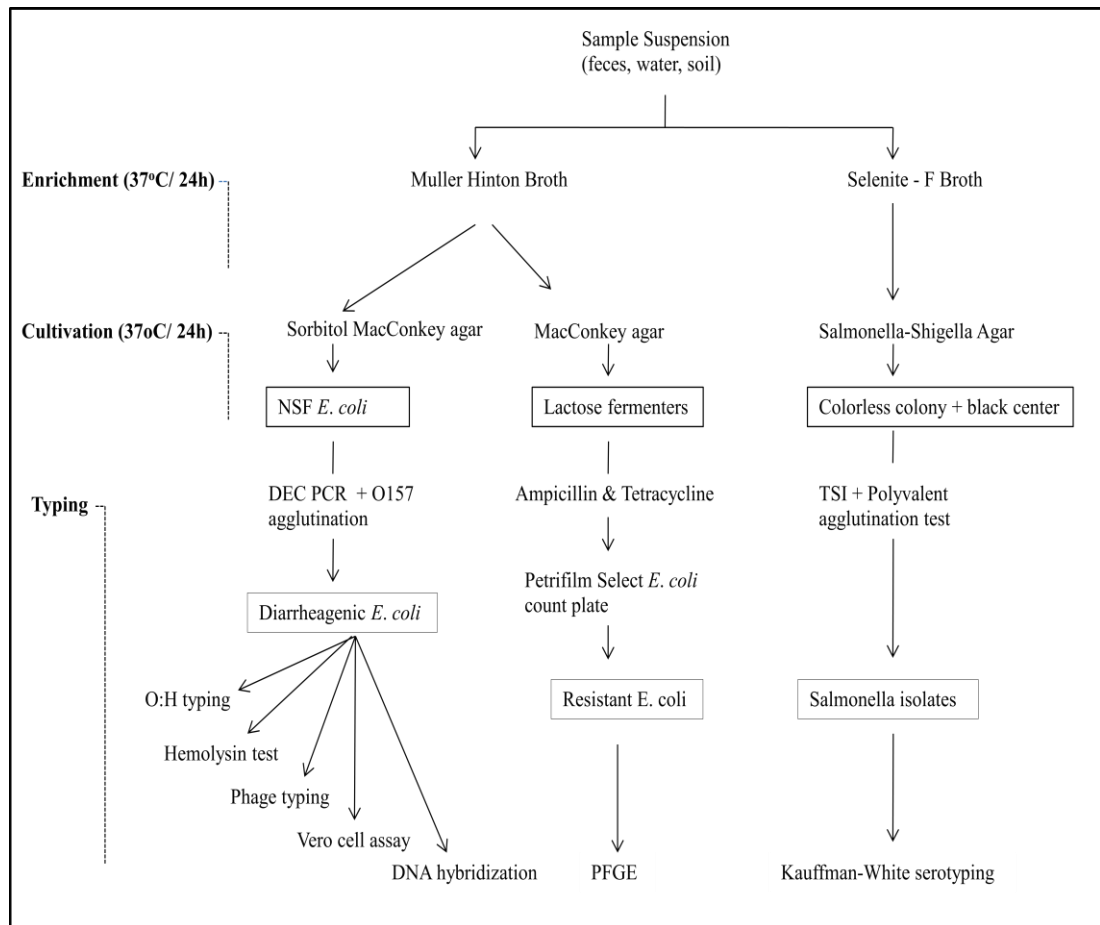


Figure 3: Schematic presentation of isolation and characterization procedures for *Escherichia coli* and *Salmonella* species

## 1.8 Thesis organization

This thesis is prepared according to “Publishable manuscript” format of Sokoine University of Agriculture. It comprises of six chapters. Chapter one introduces the research topic in an extended manner. It gives a background information, rationale, objectives and design of the study. Chapter two is a published article that addressed the first specific objective by assessing cattle and manure management practices in urban and peri-urban areas of Morogoro (Manuscript I: published in LRRD in 2012). Chapter three reports the findings to fulfill the second specific objective. Here *E. coli* was isolated and characterized from cattle farming environment. Hence, chapter three reports the occurrence and characterization of pathogenic *E. coli* from cattle, humans, soil and water (Manuscript II, intended for Vector-borne and Zoonotic diseases journal). Chapter four also addresses the second specific objective. It is a short communication on isolation of pathogenic salmonella strains such as *Salmonella amager*, *S. weltevreden*, and *S. kentucky* from humans and cattle in urban and peri-urban cattle farming environment of Morogoro (Manuscript III: intended for submission to Tanzania Journal of Health Research). Chapter five is a report that fulfills the focus of the third and fourth specific objectives. It reports the outcome of assessment of genetic relatedness of *E. coli* isolates and quantification of risk factors for transmission of these bacteria between cattle, humans, soil and water in urban and peri-urban livestock farming areas of Morogoro (Manuscript IV, to be submitted to Preventive Veterinary Medicine journal). Chapter six is the review of the epidemiology of pathogenic *E. coli* O157:H7 in Africa. It is an over view of the current epidemiological status of one of the pathogenic strain of *E. coli* that was isolated from cattle in the present study (Manuscript V, intended for submission to

the Journal of Infection in Developing Countries). Apart from the chapters, this thesis also contains a general discussion section. The general discussion section details the overall research findings that cut across individual specific objectives and gives a general overview. This section discusses matters that could not fit in individual chapters and needed collection of ideas from different chapters. There is also a general conclusion section in this thesis which gives the overall inferences of the research findings that extend beyond the focus of the individual manuscripts. Finally, the thesis has a report segment titled “perspectives and future research”. This section states general viewpoint and gives out further research areas that can yield informative and useful outcome as far as livestock farming and public health are concerned.

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## CHAPTER TWO

### 2.0 CURRENT MANURE MANAGEMENT PRACTICES AND HYGIENE ASPECTS OF URBAN AND PERI-URBAN LIVESTOCK FARMING IN TANZANIA (Manuscript I: published in LRRD, 24 (9), 2012)

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## 2.1 Abstract

The recent expansion of urban and peri urban livestock farming has resulted in close contact between animals and humans, sometimes with adverse human health effects. A survey involving 119 cattle keeping households in urban and peri-urban settings of Morogoro, Tanzania revealed that manure management practices were different from traditional practices mainly due to lack of land. Manure was collected and conveyed by using tools by 94% of respondents, while others used water or bare hands. Seventy six percent of respondents collected manure from animal houses at least once a day, a feature that was associated with housing characteristics ( $p<0.05$ ). Heaping was a common manure storage method although other cattle keepers directly spread manure on land. Manure was disposed off within residential area by 70% of respondents and this practice was associated with land area owned by or under control of the households ( $p<0.05$ ). The current manure management practices did not protect either humans, animals or the environment against the risk of contamination with potential zoonotic pathogens and therefore there is a need for the formulation of guidelines on safe manure management practices.

Keywords: environment, hygiene, manure management, peri-urban, urban

## **2.2 Introduction**

Manure is largely composed of animal excreta (faeces and urine) that is mixed up with water, beddings and secretions from nose, throat, vagina and mammary glands (Pell 1997). Recovery of pathogenic bacteria in freshly voided animal faeces shows that manure is a potential source of zoonotic pathogens contaminating the environment and represents a risk for further transmission to human (Losinger et al 1997, Pell 1997, Crump et al 2002, Guan and Holley 2003, Johnson et al 2003, Hutchison et al 2004, Hutchison et al 2005, Heuvelink et al 2007). Studies have reported cases of human gastroenteritis due to bacteria enteropathogens of animal origin following consumption of contaminated food or water or direct contact with infected animals in farms (Kapperud et al 2003, Merritt and Herlihy 2003, Hendriksen et al 2004, Smith et al 2004).

In the last few decades urban and peri-urban farming in developing countries has been progressively increasing. Its emergence and expansion not only came as a survival strategy due to reduced income and living standards (Briggs 1991, Mlozi 1996, 1997a, Mvena 1999, DFID 2002) but also as a diversification strategy to spread livelihood risks in adverse situations (DFID 2002, Simon et al 2004). Some urban and peri-urban dwellers continue to keep livestock to maintain their rural cultural values (Mlozi 1996, Mvena 1999). As a consequence of the increase, both the number of animals kept and the number of households keeping animals has increased. Urban areas of Morogoro, Tanzania, for example had a cattle population of 2,618 in 1996 (URT 1997), which almost doubly increased to 4,170 in 2006 (URT 2007). In 1984 the city of Dar es Salaam urban had 1,763 crossbred dairy cattle

(MALD 1988) but by the end of 1993 the cattle population in the urban wards of the city was reported to have increased to 14,721 (Mlozi 1997b). Rapid urban population growth and demand for animal protein has provided a boost to urban and peri-urban farming (Briggs 1991, Simons et al 2004).

Before the expansion of urban and peri-urban livestock farming, free open grazing practices required minimal effort to manage manure (Powell et al 1995, DFID 2002). Increased animal population has led to an increase in manure production in urban and peri-urban areas and hence a demand for proper handling practices. However, it is currently not known how the manure management practices have changed to adapt to densely populated areas where the space separating humans from animals and their wastes has decreased. Therefore, this study aimed at determining the current manure management practices in urban and peri-urban areas of Morogoro region of Tanzania as a basis for developing strategies to improve urban and peri-urban farming practices that safeguard human and animal health.

## **2.3 Materials and Methods**

### *2.3.1 Study area*

This study was conducted in urban and peri-urban areas of Morogoro region of Tanzania. Morogoro region typifies the relationship between dense urban populations as potential market with expanding urban and peri-urban livestock farming as source of food and income. Three districts of Morogoro region constituted the study area and included Morogoro municipality, Morogoro rural and Mvomero. Morogoro region is located between latitude 5° 58" and 10° 0" S and longitude 35°

25° and 35° 30' E. Ambient temperature ranges between 18°C and 30°C. The annual rainfall ranges between 600 mm – 1200 mm (URT 1997).

### *2.3.2 Study design and selection of households for the study*

A cross-sectional survey was conducted from February to September 2010. The study involved 119 cattle keeping households. Participants were selected from a list of 367 cattle keeping households obtained from the District Livestock Development Offices. Simple random sampling of the households was carried out by use of “rank and index” functions in Excel software. This method assigned a unique random number to each of the listed households and selected a required number of households without repetition. Five out of 119 cattle keeping households that withdrew from the investigation were replaced by a random selection of new households within the list of cattle keeping households.

### *2.3.3 Data collection and analysis*

Interviews with cattle keepers using semi-structured questionnaires and personal observations using a guide were the main tools to gather information on herd and manure management practices in the selected urban and peri-urban livestock keeping areas of Morogoro. Additionally, face to face interviews with District Livestock Development Officers about herd and manure management practices were carried out. The developed questionnaire aimed at gathering information on (1) herd characteristics and management, taking into account labor division, herd size, presence of animal species other than cattle, type of animal house roofing, floor, feeding system and history of cattle treatment; (2) manure management practices, including means, frequency and form of manure collection, storage, means and



distance of manure disposal and household area and (3) awareness on zoonotic enteropathogens. Moreover, the guided interviews with District Livestock Officers from Morogoro Municipality, Morogoro Rural and Mvomero focused on existing guidelines and their monitoring of manure handling practices in their respective areas. Some of the officers produced information materials e.g. “Environmental Sanitation By-Laws” and “Animals in Urban areas By-Laws” that give directives on animal keeping in the areas and how to deal with wastes including manure.

Data were analyzed using SPSS 15.0 such that means for continuous variables and frequency of occurrence of variable factors for categorical variables were computed. Associations between all possible combinations of categorical variables were analyzed by Pearson’s Chi-square test at significance level of 5%.

## **2.4 Results**

### *2.4.1 Herd characteristics and management*

From the observations and questionnaire, 119 research participants owned a total of 806 cattle (minimum = 1, maximum = 36, mean= 7, median= 5, SD = 5.85). Among the respondents 95.8% kept animals other than cattle within the same premises including chicken (80.7%), dogs (62.2%), goats (50.4%), pigs (27.7%), ducks (23.5%), cats (21.9%), sheep (10.9%), guinea fowls (9.2%), turkeys (5.9%), guinea pigs (1.7%), rabbits (1.7%) and monkey (0.8%).

It was noted that two different groups of people, namely family members and paid laborers were engaged in management of manure. The proportion of cattle keeping

households in the study area that used paid laborers to handle manure and execute other routine farm activities such as feeding cattle, cleaning cattle houses and milking slightly exceeded the proportion in which only family members took care of cattle (Table 1).

A large proportion of animal houses were roofed, either by thatch grass or corrugated iron sheets compared to the less popular open cattle pens (*kraal*) (Table 1). In the open cattle pens rainwater wet the soil and animals spent the nights in mud until the sun dries out the soil. This was the case in a few animal houses whose floor was made of earth in contrast to a large percentage of animal houses with concrete floor (Table 1). Only three respondents were observed to put grass on the floor of animal house as bedding material, among them, one had a house with earthed floor (Table 1). All respondents kept their animals in a confinement near to their residential area for security reasons.

The cattle were fed in different ways with less than half of cattle being kept in-door and fed by a “cut and carry” method, while the others had to move around foraging (Table 1). A relatively small fraction of cattle, mostly free range cattle used surface water such as rivers, ponds and wells while most of cattle were provided water from taps which also served the people (Table 1).

Table 1: Herd management practices among 119 urban and peri-urban cattle keepers

Variable	Category	Frequency (%)
Manure responsible person	Family member	55 (46.2)
	Paid laborer	64 (53.8)
Animal species other than cattle	Present	114 (95.8)
	Absent	5 (4.2)
Animal house roofing	Roofed	100 (84.0)
	Un-roofed	19 (16.0)
Animal house floor	Concrete	85 (71.4)
	Earth	34 (28.6)
Bedding	Present	3 (2.5)
	Absent	116 (97.5)
Animal feeding system	Zero-grazing	56 (47.0)
	Out-door	63 (53.0)
Animals' water source	Tap	71 (59.7)
	Surface water	48 (40.3)

#### 2.4.2 Manure collection, conveyance, disposal and knowledge on enteropathogens

During the night all cattle were kept in enclosures with accumulation of manure. Before discarding, manure was collected by bare hands by a few respondents with direct contact to the manure. However, the majority of respondents used utensils such as spades, hand hoes and rakes to collect manure into a pile within the animal

house. It was also observed that some respondents used a water hose to collect manure (Table 2). Irrespective of the manure collection method, people did not use any protective measures such as special clothes or gloves and were observed to have direct skin contact with manure. The majority of respondents collected manure at least once a day (Table 2).

After collection into a heap, manure was moved to storage area either by bare hands or water splash by a small number of respondents while the majority used utensils such as spade, bucket, wheel barrow, plastic bag or raw hide (Table 2). Storage of manure in heaps for some time before disposal was a common practice among many respondents although it was observed that a few of them directly spread manure from animal houses into the surrounding environment (Table 2).

Some cattle keepers used manure as fertilizer, especially those owning large pieces of land while others did not use manure as fertilizer at all. However, in both cases, most respondents spread manure direct on land as the preferred way of disposal (Table 2). Respondents who did not spread manure on land opted for burning or giving it away to friends in plastic bags. Most cattle keepers disposed the manure either as fertilizer or waste within a radius of 10 m from their residential house (Table 2).

The use of rubber boots was an observed practice by more than a half of respondents while the remaining fraction wore ordinary shoes e.g. sandals while handling manure (Table 2). There was a tendency for a large proportion of livestock keepers to allow

effluent from animal house to spread freely on the surrounding land except for a few respondents who directed the effluent into a pit (Table 2). The size of the area owned and used by households is of interest from hygiene perspective as human and animals shared such land. It was observed that respondents who had more than 1000 m<sup>2</sup> of land were in large number compared to those living on less land (Table 2)

When asked about their knowledge of pathogens associated with manure, respondents revealed that they have never heard about such pathogens except for a few respondents who were aware that there could be enteropathogens in manure that may cause enteric diseases (Table 2).

Table 2: Manure management practices among 119 urban and peri-urban cattle keepers

Variable	Category	Frequency (%)
Manure disposal method	Spread on land	108 (90.8)
	Not spread on land	11 (9.2)
Means of manure gathering	Hand picking	5 (4.2)
	Use of utensils	112 (94.1)
	Water splash	2 (1.7)
Frequency of manure collection	Once a day	72 (60.5)
	More than once a day	19 (16.0)
	Weekly	28 (23.5)
Means of manure transference	Hand picking	3 (2.6)

	Use of utensils	115 (96.6)
	Water splash	1 (0.8)
Use of rubber boots	Yes	70 (58.8)
	No	49 (41.2)
Manure treatment	Heap	99 (83.2)
	Direct spread on land	20 (16.8)
Disposal distance	Within 10m	83 (69.7)
	Outside 10m	36 (30.3)
Effluent treatment	Direct spread on land	95 (79.8)
	Pit	24 (20.2)
Household area	> 1000 m <sup>2</sup>	87 (73.1)
	≤ 1000 m <sup>2</sup>	32 (26.9)
Ever heard of pathogens in manure	No	113 (95.0)
	Yes	6 (5.0)

#### 2.4.3 Relationship between animal keeping and manure management practices

There were associations between herd characteristics and management and the way that manure was handled. For instance, the type of animal house roof was related to the type of animal house floor such that roofed animal houses had concrete floors while roofless houses had floors made of earth ( $p < 0.001$ ). These animal house characteristics were significantly associated with the frequency of manure collection from the animal houses, i.e. manure was collected at least once a day for roofed

animal houses that had a concrete floor ( $p < 0.001$ ). On the other hand, manure storage practice was associated with the size of land under control of the household. Households with an area equal or less than  $1000 \text{ m}^2$  kept manure in heaps before disposal whereas respondents with land areas more than  $1000 \text{ m}^2$  spread manure from animal houses directly onto the surrounding land ( $p = 0.015$ ). The source of water for cattle was found to be significantly associated with the type of animal feeding system. Zero grazed cattle were given tap water that was also used by humans while cattle foraging outdoor used surface water such as ponds, rivers and boreholes ( $p < 0.001$ ). When herd size was transformed into a categorical variable, it was found that herds with more than five cattle were mostly grazing outdoor while herds with five or less cattle were zero grazing ( $p = 0.009$ ).

## **2.5 Discussion**

The diverse manure management practices of the cattle keepers in the study area were determined by customs and convenience. Some farmers said that they handled the manure by the same methods since childhood; others opted for a particular manure management method because it was easy to execute. A number of farmers did not use protective measures and equipment to handle manure because of the associated costs. The differences in manure management practices and lack of hygienic protective measures among cattle keepers underlines the need for disseminating information on proper handling of animal waste to guide farmers on safe collection, conveyance, storage and disposal of manure. For instance, according to Morogoro Municipal Council (2002, 2010), manure is regarded as solid waste that is treated like any other household waste, but there does currently not exist any

guidelines or regulations on proper manure management in Morogoro region or elsewhere in Tanzania .

Manure management guidelines in other parts of the world have centred on reduction of environmental pollution, in particular eutrophication of aquatic recipients, and improvement of nutrient availability to crops. Guidelines have so far not addressed the health of personnel who handle manure at farm level or those living in areas with urban livestock. A guideline of manure management in Asia by IAEA (2008) aims at making manure handling easier, decreasing odors and water and air pollution as well as promoting production of biogas and more valuable organic fertilizer. Also manure management guidelines by Ohio State University Extension (2006) and Nova Scotia (2006) inform farmers on how to utilize manure as valuable fertilizer and energy source while at the same time protecting the environment. In general there are no guidelines in neighboring African countries that provide livestock keeper's information on sustainable manure management, in particular for urban livestock keeping. The guidelines developed for Asian, European or American farmers are of little relevance and not addressing the problems and challenges faced by livestock keepers in urban and peri-urban settings of developing countries like Tanzania (Mlozi 1996).

The current manure management practices seen in Morogoro differ from the way manure was handled a few decades ago. Before urban and peri-urban livestock farming intensified and became more commercial, manure management did not require much effort by the farmers. For instance in the city of Dar es Salaam, animals



spent daytime foraging and dropping manure everywhere, while manure accumulated during overnight confinement was left to decompose in house compounds, on live hedges or open spaces or thrown in streams or along road sides for it to be washed away by rain or sometimes applied on crop plots (Shauri 1989, Mlozi 1997b, DFID 2002). This practice happened before transition from specialization (free open grazing) to integration (confined zero grazing and crop growing), where by the former did not call for special manure handling practices compared to the latter farming system (Powell et al 1995). Reports by Mlozi (1996 and 1997b) revealed that an increased manure production in populated urban and peri-urban areas of Dar es Salaam has led to scarce area for disposal, such that decomposing manure produced odor and favored breeding of pathogens and flies. These detrimental effects of manure handling practices in urban and peri-urban areas came as a result of land scarcity and poor manure handling infrastructures because urban and peri-urban livestock farming was not integrated in planning process of towns like Morogoro, Dar es Salaam, Dodoma and Mbeya (Mvena 1999).

Animals such as cattle, sheep and goats have been reported as potential reservoirs of zoonotic pathogens most of which reside in the gastrointestinal tract and are voided in feces (Crump et al 2002, Cobbaut et al 2009, Mersha et al 2010). For instance, Kang'ethe et al (2007) isolated *E. coli* O157:H7 from cattle feces in urban and peri-urban settings of Nairobi. Cases of human infection by pathogens associated with manure due to either contact with infected animals or consumption of contaminated animal products are common (Germani et al 1997, Crump et al 2002). Manure-associated pathogens may be introduced into different places in the food production

chain. Nonga et al (2009) reported thermophilic campylobacter prevalence of 5.6% from feces of slaughtered cattle, 9.3% of dressed carcasses at abattoirs and 1.9% in beef sold in meat shops in the city of Morogoro. Other studies (Abdul-Raouf et al 1996, Benkerroum et al 2004, Kang'ethe et al 2007, Hiko et al 2008) have reported the isolation of a number of pathogens in food products of animal origin. The trend of pathogen contamination seems to build up through the food chain from animal at farm level to food products. It is evident from our results that lack of proper manure handling is associated with quite substantial faecal contamination of the environment and thus putting human and other animals at risk of infection particularly those associated with enteropathogens. Livestock keeping and manure management in peri-urban and urban areas with high densities of animals and humans demands development of guidelines and enforcement of regulations on proper hygienic manure management practices that reduce faecal contamination of the environment and protect human health

It was shown that a large proportion of respondents were not aware that manure may contain a variety of pathogens hazardous to human and animal health. Similar lack of knowledge was reported by Mlozi (1996) among livestock keepers in urban and peri-urban settings of Dar es Salaam where the farmers had little knowledge about pathogens and associated risks. Thus, it is clear that the current manure management practices of cattle keepers in Morogoro region did not aim at preventing any transmission of pathogens between human, cattle and environment or other ways to protect human and animal health.

The study by Kang'ethe et al (2007) in urban and peri-urban areas of Nairobi reported that manure handling was a risk factor for human infection and pointed out that the use of protective gear during manure handling could reduce the infection risk. Thus it is likely that the cattle keepers in the study area were at increased risk of infection because they neither wore gloves nor protective clothing during manure handling. Our findings call for further research to document the occurrence of pathogens in cattle manure, occupational health hazards for livestock keepers, their families and others living in peri-urban and urban areas where livestock are kept to establish effective guidelines and regulations that protect human health while at the same time recognizing the socio-economic benefits of urban livestock keeping.

The association between certain manure management practices and household conditions, as elucidated in this study, could be used to improve manure management practices. Improving the animal house by putting concrete floor and a roof was found important in relation to increasing the frequency of manure collection from the animal house to at least once a day. Additionally, it has been reported by Lekasi et al (2003) and Rufinol et al (2007) that improved animal houses such as that with a roof, concrete floor and good drainage reduce loss of manure and retain higher phosphorus and nitrogen content the same as when manure heaps are covered with polythene films. Therefore, improving animal housing infrastructures may not only ease manure handling workload, but is also likely to protect human, animal and environmental health while retaining the fertilizer value of the manure.

## **2.6 Conclusion**

The current manure management practices differ from those methods employed a few decades ago in both the actual practices and resource base available that is shared by human, animals and manure. Increased manure production in a shrinking space force cattle keepers to collect, convey, store and finally dispose manure. In the course of this process, human and environment are put at risk of pathogen contamination. Therefore, there is a need to design manure handling practices that suits the available land resource at the same time safeguarding human, animals and the environment.

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## CHAPTER THREE

3.0 OCCURRENCE AND CHARACTERIZATION OF SHIGA TOXIN-  
PRODUCING *ESCHERICHIA COLI* O157:H7 AND OTHER NON-  
SORBITOL FERMENTING *E. COLI* IN CATTLE AND HUMANS IN  
URBAN AND PERI-URBAN AREAS OF MOROGORO, TANZANIA  
(Manuscript II)

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### 3.1 Abstract

*Escherichia coli* strains such as Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli*, enterotoxigenic, attaching and effacing *E. coli*, and enteroinvasive *E. coli* cause diarrhea in humans. Although other serotypes exist, the most commonly reported STEC in outbreaks is O157:H7. A cross sectional study was conducted to isolate and characterize non-sorbitol fermenting *E. coli* O157:H7 from urban and peri-urban livestock settings of Morogoro, Tanzania. Human stool, cattle feces and soil and water samples were collected. Observations and questionnaire interviews were used to gather information about cattle and manure management practices in the study area. *E. coli* were isolated on sorbitol MacConkey agar and characterized by conventional biochemical tests. Out of 1046 samples, 143 (13.7%) yielded non-sorbitol fermenting *E. coli*. Serological and molecular typing of non-sorbitol fermenting *E. coli* detected 10 (7%) pathogenic *E. coli* including STEC (n=7), EPEC (n=2) and A/EEC (n=1) strains. The serotypes O157:H7, O142:H34, O113:H21, O+:H-, O+:H16 and O25:H4 were identified. One ESBL-producing isolate showed the MLST type ST131. To our knowledge, this is the first finding in Tanzania of this recently emerged worldwide pandemic clonal group, causing widespread antimicrobial-resistant infections, and adds knowledge of ST131's geographical distribution. Cattle manure was indiscriminately deposited within residential areas and there was direct contact between humans and cattle feces during manure handling. Cattle and manure management practices expose humans, animals and the environment to pathogenic *E. coli* and other manure-borne pathogens. Therefore, there is a need to improve manure management practices in urban and peri-urban areas to prevent pathogen spread and associated human health risks.

Keywords: manure, peri-urban, public health, sorbitol MacConkey agar



### 3.2 Introduction

Diarrheagenic *E. coli* such as enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) and extraintestinal pathogenic *E. coli* (ExPEC) cause different intestinal and extra-intestinal diseases in humans (Nataro and Kaper 1998; Kaper et al. 2004; Caprioli et al. 2005) including diarrhea, hemorrhagic colitis or hemolytic uremic syndrome (HUS) especially in children and the elderly (Dundas et al. 2001).

Once referred to as a rare serotype (Riley et al. 1983), non-sorbitol fermenting STEC O157:H7 is now a public health problem worldwide (Caprioli et al. 2005). STEC O157:H7 typically cause hemorrhagic colitis characterized by severe abdominal pain, watery diarrhea preceding bloody diarrhea and little or no fever (Germani et al. 1997). About 2-15% of STEC O157:H7 infections progress to HUS characterized by hemolytic anemia, thrombocytopenia and renal failure (Fitzpatrick 1999; Dundas et al. 2001). Both sporadic cases and epidemic diseases associated with STEC O157:H7 have been reported (Sang et al. 1996; Crump et al. 2002).

Hosts and sources of STEC O157:H7 are diverse and include cattle, sheep, goats, pigs, water buffalo, deer, horse, dogs, rabbits, gulls, pigeons, chicken, meat, milk, poultry and fish products, vegetables and water (Caprioli et al. 2005; Ferens and Hovde 2011). Cattle are the main reservoir (Chase-Topping et al. 2008) and have been associated with several cases of human infection (Aspán and Eriksson 2012). Adult cattle are tolerant to infection due to lack of vascular receptors for the Shiga toxins produced by STEC O157:H7 (Pruimboom-Brees et al. 2000) whereas iliocolitis in calves following experimental infection is fatal (Dean-Nystrom et al.

1998). Transmission of STEC O157:H7 to humans occurs through the fecal-oral route following ingestion of contaminated food or water (Tuttle et al. 1999; Olsen et al. 2002) or direct contact with contaminated surfaces or animals (Crump et al. 2002).

Urban and peri-urban livestock farming is expanding in developing countries primarily due to high demand for animal protein to serve rapidly growing urban populations, but also to generate household income (Mlozi 1997). In Tanzania, there is an increase in both the number of cattle and households engaged in cattle keeping with such households located in close proximity to households not keeping cattle. Thus, cattle and other livestock manure is deposited indiscriminately in and on the fringes of cities and contaminate the external environment, e.g. cattle defecate when taken around during the day for feeding or when manure is poorly managed at household level (Lupindu et al. 2012). However, in contrast to well-known health risks associated with poor management of human excreta little is known about health risks associated with livestock keeping and manure management in urban areas.

The present study, therefore, aimed to estimate the prevalence of NSF STEC O157:H7 and other NSF *E. coli* in cattle, humans and the associated environment of the municipality of Morogoro, Tanzania.

### **3.3 Materials and Methods**

#### *3.3.1 Selection of study subjects and sample collection*

The study included smallholder cattle keeping and non-cattle keeping households of urban and peri-urban areas of Morogoro municipality, Tanzania. A herd of cattle belonging to a household was regarded as a cluster. To establish the

prevalence of non-sorbitol fermenting (NSF) STEC O157:H7 the number of clusters was calculated (Bennett et al. 1991) and adjusted as the population of clusters from which the sample was drawn was small (Thrusfield 1995). An estimated prevalence (P) of NSF STEC O157:H7 was set at 50% (Thrusfield 1995), the intra-cluster correlation coefficient ( $\rho$ ) at 0.2 (Otte and Gumm 1997) and significance level was set at 5%. The average number of cattle in a cluster was found to be five during a preliminary survey and thus used in calculation of number of clusters. One hundred cattle keeping households, who kept 446 cattle in total, were randomly selected from a sampling frame of 367 households and matched to their respective non-cattle keeping neighbors living within a radius of 100 meters. Stool from one member of a cattle keeping household involved in cattle and manure management and any member from a non-cattle keeping household, was collected early in the morning following prior notification and provision of a stool collection container the preceding evening. Water and soil samples were collected from the household the same morning that stool was collected. Soil samples (2-5 cm top soil) were collected from five different locations of the household premises and mixed to form a pooled soil sample of about 100 g. Surface water such as borehole or pond or any other available water source for human and/ or cattle consumption at household level was collected in sterile 250 ml bottles for isolation of *E. coli*. Individual fecal samples of 100-150 g were collected by hand from the rectum using a disposable plastic glove from all cattle kept in a household. All samples were immediately placed in an insulated box with cooling elements and transported to the laboratory where bacteriological analysis was initiated. Some samples were kept overnight at 4-5°C for analysis the following day with such samples being stored for a maximum of 24

hrs. A total of 200 human stool, 200 soil, 200 water and 446 fecal samples from cattle were collected for *E. coli* analysis between December 2010 and February 2012. Bacteria isolation was done at Sokoine University of Agriculture, PCR was conducted at University of Copenhagen while Vero cell assay and DNA hybridization was carried out at Staten Serum Institute, Denmark.

### 3.3.2 Isolation of *E. coli*

One gram of stool, soil and feces and one milliliter of water sample were mixed with four ml of normal saline (0.9% NaCl) to form a sample suspension. One ml of the sample suspension was enriched by incubation at 37°C for 24 hrs in four ml of brain infusion broth (CM0225, Oxoid, Hampshire, UK). Sample enrichments were subcultured onto Sorbitol MacConkey agar (CM0813, Oxoid) supplemented with cefexime-tellurite (SR0172E, Oxoid) and incubated at 37°C for 24 hrs. Medium sized, round, smooth, colorless (non-sorbitol fermenting) colonies were checked for purity after subculture onto blood agar plates and then tested for lactose fermentation on MacConkey agar (M082-500G, Himedia, Mumbai, India),  $\beta$ -glucuronidase and  $\beta$ -galactosidase activities on Brilliance *E. coli* agar (CM1046, Oxoid) and tryptophanase activity in the indole test.

All non-sorbitol fermenting *E. coli* isolates were subsequently tested for the presence of O157 somatic antigen by agglutination test using Wellcolex *E. coli* O157 kit (Remel Europe Ltd, UK) according to manufacturer's instruction.

### 3.3.3 Detection of diarrheagenic *E. coli*

Multiplex PCR for diarrheagenic *E. coli* was done for all non-sorbitol fermenting *E. coli* isolates using a DEC PCR kit (SSI Diagnostica, Hillerød,

Denmark) to check for presence of the following genes; intimin gene (*eae*) with amplicon size 377 bp; verocytotoxin 1 (*vtx1*) with amplicon size 260 bp; verocytotoxin 2 (*vtx2*) with amplicon size 420 bp; heat stable enterotoxin, human variant (*estA*-human) with amplicon size 151 bp; heat stable enterotoxin, porcine variant (*estA*-porcine) with amplicon size 160 bp; heat labile enterotoxin (*eltA*) with amplicon size 479 bp; invasive plasmid antigen (*ipaH*) with amplicon size 647 bp and 16S rDNA genes with amplicon size 1062 bp. The latter gene served as a positive PCR internal control. Template DNA preparation, PCR master mix and PCR reaction volume were prepared according to manufacturer's instruction. PCR amplification was run in a thermocycler at the following conditions; initial denaturation at 95°C for two min followed by 35 cycles of denaturation at 94°C for 50 sec, annealing at 62°C for 40 sec and extension at 72°C for 50 sec. The final extension was done at 72°C for three min. For each sample and the controls, 18 µl of PCR product was run in separate wells on 2% agarose gel at 125 V for one hour followed by staining in ethidium bromide (SSI-Diagnostica 2011). STEC isolates were sub-typed by PCR for *vtx1* and *vtx2* genes (Scheutz et al. 2012).

Vero cell cytotoxicity assay was performed for all isolates positive for virulence genes to test for cytopathic effects on Vero cell monolayers (Scheutz 1997).

Strains found to harbor virulence genes in the DEC PCR were confirmed by dot blot DNA hybridization and tested for additional virulence genes at the Statens Serum Institut, Denmark. DNA probes included *vtx1*, *vtx2*, *eae*, *ehxA*, EAF, *bfpA*, *saa*, *astA* and *vtx2f*. Isolates were allowed to grow on the nylon membrane positioned

on top of an agar plate. The colonies were lysed, denatured and neutralized using standard conditions and then hybridized (Scheutz 1997).

#### 3.3.4 *Serotyping of diarrheagenic E. coli*

O and H antigen serotyping was performed according to standard procedures at the Statens Serum Institut (Ørskov and Ørskov 1984; SSI 2011). In summary, boiled cultures of isolates were tested against pooled O antisera and single O antisera of respective pools. Infusion broth inoculated with a colony of isolate was incubated at 37°C for 24 hours. Broth cultures were boiled at 100°C for one hour and left to cool on the table for one hour before use. Eighty microliters of pooled O antisera was mixed with an equal amount of boiled culture in microtiter plates and incubated in humid atmosphere at 50°C overnight. A boiled culture positive for pooled O antisera was tested with an equal amount of single O antisera and incubated at 50°C overnight. Positive reaction, agglutination covering the bottom of microtiter well, was read against artificial light on a black background.

For H antigen typing, one colony from agar medium was inoculated in semi-solid medium and incubated at 37°C. Motile cultures were passed to another semi-solid medium using a Pasteur pipette and incubated overnight at 37°C. Cultures were further passed to nutrient beef broth, incubated at 37°C for 6 hrs in a roller device and fixed with formaldehyde 0.5%. One hundred eighty microliters of pooled H-antiserum were mixed with an equal amount of fixed culture in a glass tube. The reaction was read after two hours incubation of the mixture at 50°C. Positive fixed cultures were tested with an equal amount of single H antisera of respective H pool. A positive agglutination reaction was fluffy.

### 3.3.5 Antimicrobial susceptibility test

Antimicrobial susceptibility testing of all non-sorbitol fermenting *E. coli* isolates was determined at University of Copenhagen by the disc diffusion method according to standards of the Clinical Laboratory Standard Institute (CLSI, 2008). The following discs (Oxoid, UK) were used: amoxicillin (30 µg), ampicillin (10 µg), sulphamethazole/trimethoprim (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), streptomycin (10 µg) and cefotaxime (30 µg). Fresh colony suspension, equivalent to 0.5 McFarland standards, was tested against the antimicrobial discs on Mueller-Hinton agar and the diameters of the zone of complete inhibition were read after 18 hrs of 37°C incubation. Isolates were also tested for presence of Extended Spectrum  $\beta$ -Lactamase (ESBL) genes for the TEM, SHV, OXA-10 group, and the CTX-M group 1, 2, 8 and 9 enzymes by PCR as previously described (Hansen et al. 2012). The O25-ST131 clone allele-specific PCR for the *paB* gene was performed as previously described (Clermont et al. 2009).

### 3.3.6 Ethical approval

The study was approved by the Tanzania National Institute for Medical Research (NIMR) Ethical Board (NIMR/HQ/R 8a/Vol. IX/927). Confidentiality of the study outcome and all conditions for research approval were observed throughout the study

## 3.4 Results and Discussion

### 3.4.1 Non-sorbitol fermenting *E. coli*

From the total of 1046 samples analyzed, 143 yielded non-sorbitol fermenting *E. coli* including 95 isolates from cattle feces, 33 from human stool, eight from soil and seven from water samples. Four isolates (4.2%) from cattle were positive for

O157 somatic antigen. Traditionally, the inability of *E. coli* O157:H7 to ferment sorbitol has been utilized to differentiate them from other strains. The use of sorbitol MacConkey agar to screen for STEC O157:H7 is reported to miss out some non-motile VTEC O157 strains because they can ferment sorbitol (Karch and Bielaszewska, 2001), however, recovery of STEC (including non-O157), EPEC and A/EEC in the present study (Table 1) illustrates that there is a broad range of diarrheagenic *E. coli* that do not ferment sorbitol.

Multiplex DEC PCR of all non-sorbitol fermenting *E. coli* revealed that 11 isolates (7.7%) carried at least one virulence gene (Fig. 1). The proportion of cattle carrying non-sorbitol fermenting diarrheagenic *E. coli* was 10.5% while that of water samples positive for NSF diarrheagenic *E. coli* was 14.3%. An amplicon was produced for 16S rDNA in all tested isolates except for the negative control confirming that the PCR was done correctly. The results from the multiplex DEC PCR, DNA hybridization and O:H serotyping of the non-sorbitol fermenting *E. coli* are shown in Table 1. Isolates contained either one or a combination of *vtx1*, *vtx2*, *eae*, *ehxA*, EAF, *bfpA* and *astA* genes. Four isolates carried both *eae* and *vtx2* genes, two isolates carried *vtx1* and *vtx2* and two isolates carried EAF and *bfpA* genes. Six out of 11 diarrheagenic *E. coli* isolates had cytotoxic effects on Vero cells (Table 2). The O157:H7 was the most commonly isolated serotype (four isolates), while other isolates were O113:H21, O142:H34, O+:H- and O+:H16. One STEC isolate was non-motile and two isolates had an O antigen that could not be determined (Table 1). The *vtx* subtypes were dominated by *vtx2c* (five out of seven isolates) (Table 2). Three bovine isolates had more than one subtype of the *vtx* gene while one isolate contained three *vtx* subtype genes (Table 2).



Occurrence of genes *vtx1*, *vtx2*, *eae*, *ehxA* and *astA* in different combinations and the cytopathic effect of isolate extracts on Vero cells suggest that these isolates can cause disease in humans (Boerlin et al. 1999; Blanco et al. 2003; Caprioli et al. 2005). Different *vtx2* subtypes were found in the current study, a finding also reported earlier (Scheutz et al. 2001). Virulence genes *eae* and *vtx2* have been more often associated with serotypes reported from human diseases than *vtx1* (Boerlin et al. 1999). Moreover, specific subtypes of *vtx2* have been associated with HUS cases whereas other subtypes seem to be most commonly associated with uncomplicated diarrhea (Friedrich et al. 2002). Our recovery of *vtx1a*, *vtx2b*, *vtx2c* and *vtx2d* in different combinations and in some isolates in combination with the *eae* gene shows that inadequate management of cattle manure and associated human exposures may represent health risks for people living in urban areas with livestock keeping.

#### 3.4.2 Prevalence of STEC

The prevalence of STEC O157:H7 in cattle was 0.9% (95% CI: 0.29 – 2.15) while the prevalence of STEC in cattle was 1.6% (95% CI: 0.69 – 3.08). The overall prevalence of diarrheagenic *E. coli* in cattle feces was 2.2% (95% CI: 0.99 – 3.67) and 0.5% (95% CI: 0.025 – 2.44) in water. Each individual diarrheagenic *E. coli* isolate came from a different source, in which case nine isolates originated from different cattle and herds and one isolate was isolated in water sample in a household where diarrheagenic isolates could not be recovered from either human stool, cattle feces or soil samples. There may be little transmission within clusters, but these scattered multiple sources increase the chance of pathogen- transmission to humans,

animals and the environment in close vicinity to the clusters positive for diarrheagenic *E. coli*.

The low prevalence of NSF STEC O157:H7 found in cattle in Morogoro is similar to what has been reported in South Africa (1.3%) (Sargeant et al. 2000) and in Kansas, United States for dairy cattle (0.28%), beef cattle on pasture (0.71%) and beef cattle in feedlots (0.33%) (Hancock et al. 1994). However, higher occurrence has been reported elsewhere in South Africa where 5.4-20% of cattle were positive for STEC O157:H7 (Ateba and Bezuidenhout 2008). The prevalence of 1.6 % STEC in cattle in the present study is comparable to what has been reported in Spain in adult healthy cattle (2.5%) but lower than that in calves (7.9%) and heifers (20.2%) in the same country (Orden et al. 2002). There are no previous records for the prevalence of STEC O157:H7 in cattle in Tanzania and neighboring countries. Thus, the estimated prevalence of STEC O157:H7 was set at 50% to maximize the sample size when the sample size was calculated (Thrusfield 1995). However, non-O157 STEC has been isolated from both cattle and children in neighboring Uganda (Majalija et al. 2008) and a study on *E. coli* O157:H7 in human patients with diarrhea in Morogoro, Tanzania, found that 0.7% of strains harbored *vtx1* or *vtx2* as well as the *eae* gene (Raji et al. 2008). It remains to be investigated whether EPEC strains in cattle feces and water sources as documented in this study may be associated with human diseases, e.g. gastroenteritis in children due to EPEC infection that has been reported in Dar es salaam, Tanzania where livestock is also kept in urban areas (Moyo et al. 2011). Our results document for the first time that STEC (including *E. coli* O157:H7), EPEC and A/EEC strains are present in cattle and water sources in Tanzania. However, if the use of sorbitol MacConkey agar to recover NSF *E. coli*

can detect DEC at this rate, there arise questions about the magnitude of the prevalence of sorbitol fermenting diarrheagenic *E. coli* and sorbitol fermenting O25:H4, ST131 in this environment.

#### 3.4.3 Antimicrobial resistance

The diarrheagenic *E. coli* isolates were all susceptible to amoxicillin, ampicillin, sulphamethazole/trimethoprim, tetracycline, ciprofloxacin, gentamycin, streptomycin and cefotaxime. However, a non-diarrheagenic isolate from human (O25:H4) (Table 1) tested positive as O25b-ST131 by the clone allele-specific PCR for the *paB* gene which is identical to the worldwide Extended Spectrum  $\beta$ -Lactam (ESBL) producing clone, and was positive for CTX-M-gr. 1 gene. ESBL variant TEM-63 and CTX-M -15 have previously been isolated from blood cultures of patients in Dar es Salaam, Tanzania (Blomberg et al. 2005) and the isolation of O25:H4, ST131 from the stool of a healthy individual in the current study gives another dimension of concern of resistant gene spread among the population.

#### 3.4.4 Cattle and manure management practices

Cattle and manure management practices for diarrheagenic *E. coli* positive herds are summarized in Table 3. In general, cattle at the study sites and elsewhere in peri-urban areas of Tanzania, roam around and different herds are allowed to mix while foraging during daytime and are then housed at night within residential areas. The manure accumulated overnight are collected by bare hands, spades and wheelbarrow and disposed of as fresh manure in and around residential areas. The effluent from the animal house was indiscriminately discharged and leached into the

surrounding land in all households with cattle positive for diarrheagenic *E. coli*. In all nine households with cattle positive for diarrheagenic *E. coli*, humans and cattle were found to share common water sources for drinking purposes (Table 3).

During grazing cattle may have contact with other animals including wildlife. The indiscriminate defecation on grazing land and at household premises increase exposure and spread of pathogens between animals, humans and the environment, e.g. children playing around household perimeter are likely to have high exposure to animal manure through contact with contaminated soil. Manure effluents are also discharged from cattle houses resulting in further contamination of the surrounding land with cattle keepers and their household members being at increased risk of exposure to diarrheagenic *E. coli*. At the study sites, cattle and households often share source of surface water such as rivers and ponds, especially during dry season when these are important common sources of drinking water. Close contact between human and livestock as documented in this study has been reported to result into sharing of non-pathogenic commensal bacteria between humans and different animal species (Rwego et al. 2008). Given such interactions, presence of pathogenic microbes can lead to either human or animal infection or environmental contamination. For instance, outbreak of enteric infectious diseases, including those due to *E. coli* O157:H7 have been reported among people on farm visits in USA who had direct contact with manure from infected cattle (Crump et al. 2002; Smith et al. 2004). However, it is uncertain to what extent the occurrence of diarrheagenic *E. coli* found in cattle and in water in the present study represents real human health hazards. Therefore, there is a need for further research to quantify possible risk factors for pathogen transmission from animals or environment to animal keepers

and neighboring community in the study area and recommend appropriate measures to safeguard public health.

### **3.5 Acknowledgement**

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### **3.6 Author Disclosure Statement**

No competing financial interests exist.

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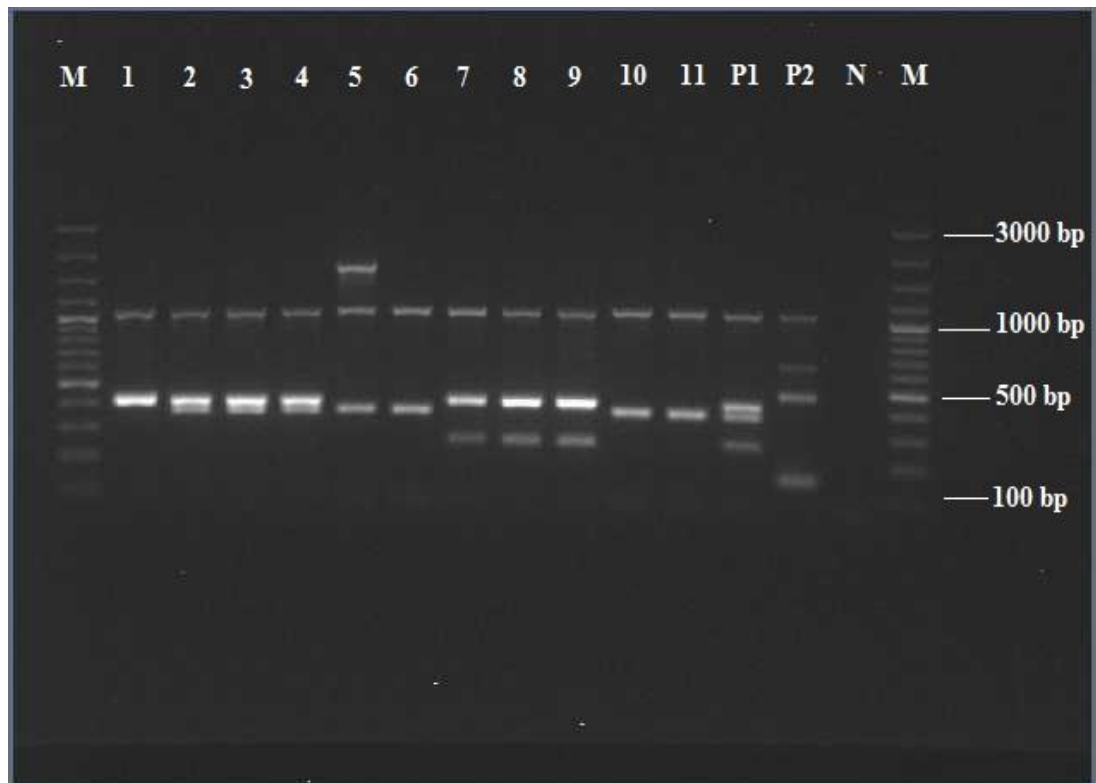


Figure. 1. Multiplex DEC PCR for non-sorbitol fermenting *E. coli* isolates: lanes M: molecular weight size marker (100-bp plus DNA ladder); lane 1: BMZU001 (*vtx2*); lanes 2: BMKB070 (*vtx2* and *eae*); lane 3: BMKB068 (*vtx2* and *eae*); lane 4: BMKB069 (*vtx2* and *eae*); lanes 5: BKIN069 (*eae*); lane 6: BKIH015 (*eae*); lane 7: BKIH040(2) (*vtx1* and *vtx2*); lane 8: BKIH101 (*vtx1* and *vtx2*); lane 9: BBIG020(1) (*vtx1* and *vtx2*); lane 10: BKIL032 (*eae*); lane 11: WKIL019 (*eae*); lane P1: positive control for *vtx2*, *eae* and *vtx*; lane P2: positive control for *ipaH*, *eltA* and *estA*; lane N: negative control.

Table 1. Virulence genes and subtypes of diarrheagenic non-sorbitol fermenting *E. coli* isolated from cattle, humans, soil and water in Morogoro Municipality, Tanzania.

Sample ID	Source	Virulence genes							Serotype	Pathotype
		<i>vtx1</i>	<i>vtx2</i>	<i>eae</i>	<i>ehxA</i>	EAf	<i>bfpA</i>	<i>astA</i>		
BMZU001	bovine	-	+	-	-	-	-	-	O113:H21	VTEC
BMKB068	bovine	-	+	+	+	-	-	+	O157:H7	VTEC
BMKB069	bovine	-	+	+	+	-	-	+	O157:H7	VTEC
BMKB070	bovine	-	+	+	+	-	-	+	O157:H7	VTEC
BKIN069	bovine	-	+	+	+	-	-	+	O157:H7	VTEC
BKIH015	bovine	-	-	+	+	-	-	+	O+:H-	A/EEC
BKIH040(2)	bovine	-	-	-	-	-	-	-	O49:H30	
BKIH101	bovine	+	+	-	-	-	-	-	O+:H16	VTEC
BBIG020(1)	bovine	+	+	-	-	-	-	-	O113:H21	VTEC
BKIL032	bovine	-	-	+	-	+	+	-	O142:H34	EPEC
WKIL019	water	-	-	+	-	+	+	-	O142:H34	EPEC
SBIG022	soil	-	-	-	-	-	-	-	O+:H33	
HBIG020	human	-	-	-	-	-	-	-	O25:H4	
HBIG021	human	-	-	-	-	-	-	-	O83:H31	
HBIG010	human	-	-	-	-	-	-	-	O+:H33	
BBIG089	bovine	-	-	-	-	-	-	-	(O20):H-	
BBIG036	bovine	-	-	-	-	-	-	-	O8:H9	

Table 2. Vero cell assay (VCA) and *vtx* subtyping for non-sorbitol fermenting diarrheagenic *E. coli* isolates.

Sample ID	Serotype	Source	VCA	<i>vtx1</i>	<i>vtx2</i>	<i>vtx</i> subtypes
BKIH101	O+:H16	bovine	+	+	+	<i>vtx1a</i> ; <i>vtx2c</i>
BKIN069	O157:H7	bovine	-	-	+	<i>vtx2c</i>
BMKB070	O157:H7	bovine	+	-	+	<i>vtx2c</i>
BMKB068	O157:H7	bovine	+	-	+	<i>vtx2c</i>
BMKB069	O157:H7	bovine	+	-	+	<i>vtx2c</i>
BMZU001	O113:H21	bovine	+	-	+	<i>vtx2b</i> + <i>vtx2d</i>
BBIG020(1)	O113:H21	bovine	+	+	+	<i>vtx1a</i> ; <i>vtx2b</i> + <i>vtx2d</i>

Table 3. Cattle and manure management practices by households with diarrheagenic *E. coli* positive herds (n=9).

Parameter	Practice	Number of practicing herds
Cattle feeding	Communal grazing	7
	Zero grazing	2
Manure disposal location	Disposal within residential area	5
	Disposal outside residential area	4
Manure disposal form	Spread of fresh manure on land	8
	Spread of compost on land	1
Animal house effluent disposal	Leaching to surrounding areas	9
Manure collection method	Pit collection	0
	Use of bare hands, spades and wheel barrow	9
Drinking water source	Surface water sharing between cattle and people	9

## CHAPTER FOUR

4.0 ISOLATION OF *SALMONELLA AMAGER* AND OTHER ZOOONOTIC  
SALMONELLA SEROTYPES FROM HUMANS AND CATTLE IN  
MOROGORO TANZANIA (Manuscript III: Short communication)

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Salmonellosis in humans is a public health challenge worldwide (Herikstad et al., 2002). Human infection is reported to be the result of consumption of contaminated food (Domingues et al., 2012) and non-food transmission routes such as contact with infected animals or contaminated environment (Herikstad et al., 2002). Commonly, salmonella are harbored by domestic animals (Heuvelink et al., 2007), wildlife (Smith et al., 2002) and the environment (Johnson et al., 2003). Human non-typhi salmonellosis are in a form of outbreaks (Hanning et al., 2009; King et al., 2011) but sporadic cases take a big share of the health challenge (Domingues et al., 2012). Some pathogens that have a history of causing disease outbreaks in previous years may seem not to be a public health threat any more e.g. *S. amager* (Beliakov et al., 1969; Vernon, 1969), but the isolation of these bacteria in unusual circumstances draws attention. This is especially when there are reports of emerging and re-emerging diseases due to changes in environmental, ecological and microbial factors (Myers et al., 2012). The present short communication reports first time isolation of *Salmonella amager* from asymptomatic human subject in cattle keeping environment in Tanzania.

Two hundred households living in urban and peri-urban cattle keeping areas of Morogoro participated in the study. Approval was granted by the Tanzania National Institute for Medical Research (NIMR) Ethical Board (NIMR/HQ/R 8a/Vol. IX/927) after meeting all research ethical requirements. Stool, water, soil and cattle fecal samples from each participating household were collected from December 2010 to February 2012. A total of 1046 samples, of which 200 were stool, 200 were water, 200 soil and 446 rectal cattle feces were processed for the



purpose of isolating zoonotic salmonella strains. One gram of stool, cattle fecal, soil and one milliliter of water sample was mixed with 4 ml of normal saline and 1 ml of the sample suspension was enriched by overnight incubation in selenite fecal broth at 37°C. The bacteria growth was subcultured on Salmonella-Shigella agar at 37°C for 24 hrs. Colourless colonies with a black center were biochemically tested by urease and lysine carboxylase tests. Urease negative and lysine carboxylase positive colonies were tested against Salmonella polyvalent agglutinating sera (REMEL30858201 ZC02 – LOT 820883) and serotyped by Kauffmann-White M03-03-001 method at Danish Institute for Technology (DTU). Confirmed salmonella isolates were tested for antimicrobial susceptibility against ampicillin (10 µg), sulphamethazole/trimethoprim (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), streptomycin (10 µg) and cefotaxime (30 µg) (Oxoid, UK) by disc diffusion method according to standard procedure (CLSI, 2008).

Out of 21 colourless isolates with a black center, six were positive for agglutination test against polyvalent antisera and four were confirmed salmonella strains as shown in the table 1. The overall isolation rate of Salmonella was 0.38%. This low isolation rate of Salmonella from human stool (1%) and cattle feces (0.45%) and none from soil and water samples could be due to the selective enrichment step effectiveness, whereby selenite fecal broth has been reported to perform poorly (Michael et al., 2003). *Salmonella amager* was resistant to ampicillin and tetracycline. *S.*

*weltevreden* was susceptible to all antimicrobials except ampicillin while *S. kentucky* isolates were susceptible to cefotaxime and gentamicin.

Table 1: Confirmed Salmonella isolates from cattle and human samples

SN	Isolate Identity	Source	Salmonella strain	DTU case number
1	HMKS001	human	<i>Salmonella amager</i>	2011-60-2692
2	HBIG008	human	<i>Salmonella weltevreden</i>	2012-60-1136
3	BKIL002(1)	bovine	<i>Salmonella kentucky</i>	2012-60-1136
4	BKIL002(2)	bovine	<i>Salmonella kentucky</i>	2012-60-1136

All salmonella isolates showed resistance to ampicillin, while *S. amager*, *S. kentucky* were also resistant to tetracycline. These are inexpensive drugs that are used as first line treatment option in Tanzania (Moyo et al., 2010). In the present study *Salmonella weltevreden* was isolated from an asymptomatic human being but it is well reported cause of human gastroenteritis (Thong et al., 2002; Emberland et al., 2007; D'Ortenzio et al., 2008). *Salmonella kentucky* causes gastroenteritis in humans (Weill et al., 2006; Bonalli et al., 2012) and the isolation from cattle in this study supports evidence that cattle may act as a source for human infection. Outbreak of human gastroenteritis due to *Salmonella amager* was reported in Russia and UK five decades ago (Beliakov et al., 1969; Vernon, 1969). Since then there has been no reported cases of human gastroenteritis due to *Salmonella amager* but the bacteria has been isolated from the well-known reservoir, free-flying Peregrine Falcons in Sweden in recent years (Palmgren et al., 2004). The isolation of *Salmonella amager* in the present study is a sign that the bacteria still exists in the environment and

recovery of the bacteria from an asymptomatic human subject call for further research with regard to its epidemiology.

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## CHAPTER FIVE

5.0 TRANSMISSION OF ANTIBIOTIC-RESISTANT *E. COLI* BETWEEN CATTLE, HUMANS AND ENVIRONMENT IN CLOSE HUMAN-LIVESTOCK PROXIMAL FARMING SYSTEM IN URBAN AND PERI-URBAN AREAS OF MOROGORO, TANZANIA (Manuscript IV)

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## 5.1 Abstract

Urban and peri-urban livestock farming is expanding world-widely because of increased urbanization. This poses a public health risk to zoonotic diseases, as livestock are reservoirs of several zoonotic pathogens. In an attempt to quantify this perspective, this study, involving 100 clusters where cattle were kept in close proximity of humans in urban and peri-urban areas of Tanzania, was conducted. A total of 1046 fecal and environmental samples were cultured on ampicillin and tetracycline soaked Petri film Select *E. coli* count plate. One hundred eighteen ampicillin and tetracycline resistant *E. coli* (40 from human stool, 50 from cattle feces, 21 from soil and seven from water samples) were isolated from 44 different clusters. Pulsed-field gel electrophoresis (PFGE) of *Xba*I digested chromosomal DNA was used to compare the genetic relatedness of the ampicillin and tetracycline resistant *E. coli* isolates. Indistinguishable band patterns of the ampicillin and tetracycline resistant *E. coli* isolates from 23 of 44 (52%) clusters that fell under eight distinct clonal complexes was revealed. This suggests that transfer of bacteria between cattle, humans, water and soils within and from cattle farms to neighbors occurred frequently. Logistic regression revealed that animal housing infrastructure (Odd Ratio 19.2) and manure disposal distance (Odd Ratio 0.4) were associated with bacteria transmission within and between clusters. Animal husbandry and management (including manure handling) should be improved to reduce risks of transmission of enteropathogens between and among livestock and humans.

Keywords: urban livestock farming, *E. coli*, genetic relatedness, PFGE

## 5.2 Introduction

The world is experiencing a continuous increase in urbanization (Seto et al., 2010) and the livestock sector is trying to keep pace with the changes (Thornton, 2010). Particularly in developing countries this has led to increased urban and peri-urban livestock farming to feed the urban population and generate household income (Mlozi, 1997). This may pose a risk to public health, because livestock are reservoirs of several zoonotic enteric pathogens.

The current study dealt with cattle as an example of urban and peri-urban livestock in order to quantify health risks associated with the enterprise. In such a system cattle are either kept in door all the time under intensive system or go around foraging during the day time exposing them to other animals from different herds under semi-intensive system (Lupindu et al., 2012). In the latter group cattle defecate haphazardly while grazing and so deposit pathogens to the pastures, soils or water around, or they can pick pathogens and take them to residential areas where they are housed at night. In both management systems, direct contact of humans with infected animal or feces may lead to human infection (Smith et al., 2004) while disposal of fresh animal feces on land may cause environmental contamination and possibly human infection (Nygård et al., 2004). Chances of human infection increases when infected animals share drinking water with humans (Kusiluka et al., 2005).

Many species of pathogens reside in the intestines of animals such as cattle and are shed through feces (Heuvelink et al., 2007; Klein et al., 2010). Therefore, the ways animals and manure are managed are important factors in transmission of the pathogens from animals to the environment and or humans (Pell, 1997; Hutchison et al., 2005). Human enteric diseases due to pathogens such as *E. coli*, *Salmonella*,



*Campylobacter* and *Cryptosporidium* that are harbored by animals and the environment are a common public health challenge (Howie et al., 2003; Smith et al., 2004; Heuvelink et al., 2007). The common modes of pathogen transmission include direct or indirect contact with infected animals or contaminated environment (Crump et al., 2002; Smith et al., 2004). Vehicles such as water (Olsen et al., 2002; Johnson et al., 2003) and food products (Benkerroum et al., 2004) are well documented means of pathogen transmission.

Investigations for the presence of pathogens are normally conducted following disease outbreaks (Howie et al., 2003; Smith et al., 2004). In the absence of disease syndromes, the interaction of humans with animal and environment may yet lead to transmission of microorganisms (Silvestro et al., 2004). This transmission may happen between animals, cattle keepers and their non-cattle keeping neighbors especially in densely populated urban and peri-urban areas. The present study aimed at assessing how frequently this happens by using *E. coli* isolates from humans, cattle, soil and water from livestock farming in urban and peri-urban areas in a selected study area in Morogoro region, Tanzania, as indicator organisms.

### **5.3 Materials and methods**

#### *5.3.1 Study site and selection of cattle keeping households*

The study was conducted in urban and peri-urban areas of Morogoro, Tanzania and included 100 cattle keeping households randomly selected in a prevalence study of diarrheagenic *E. coli* (Lupindu et al., submitted). For each cattle keeping household, a non-cattle keeping neighbor household was selected from within a radius of 100 m and the two households were considered a cluster.

### 5.3.2 *Stool, cattle feces, water and soil sampling*

Approximately 200 ml of water samples were collected at the household and the water source was borehole, pond, river or stagnant rain water used by livestock and/ or humans for drinking purposes. Furthermore, a pooled soil sample of approximately 100 g was obtained by mixing top 2-3 cm soil from different random areas of the household premises. Stool sample from cattle-keeping household stool was collected from a persons involved in cattle and manure management while from the non-cattle keeping household stool sample was collected from any member. A person provided with a stool collection container went into a toilet and collected his or her fresh stool, at the same time other sample types were collected. Moreover, approximately 100 g of individual fecal samples were collected with a gloved hand from the rectum of all cattle in the households. All collected samples were immediately placed in an insulated container with ice packs and transported to the laboratory where processing and microbiological analysis was initiated on the same day. The sample collection process was done from December 2010 to February 2012 whereby 200 stool, 200 soil, 200 water and 446 cattle fecal samples were collected.

### 5.3.3 *Isolation of E. coli and antimicrobial susceptibility testing*

Human stool, cattle fecal and soil samples were made into suspension by adding 1g of each sample type with 4 ml of normal saline and mixed by vortexing. For water samples, 1 ml was mixed with 4 ml of normal saline and vortexed. A loopful of sample suspension was inoculated onto MacConkey agar (VM327265, Merck, Darmstadt, Germany) and incubated at 37°C for 24 hrs. Smooth, medium sized suspected *E. coli* colonies were picked for further characterization.

*E. coli* suspected isolates were confirmed and tested for resistance to ampicillin and tetracycline by spotting bacterial suspensions made from normal saline on Petri film Select *E. coli* count plate (2012-10 KA, 3M Microbiology Products, USA). Antimicrobial stock solution was prepared by dissolving 32 mg ampicillin and 64 mg of tetracycline in one liter of distilled sterile water. One milliliter of antimicrobial solution was placed onto bottom film of the Petri film Select *E. coli* count plate and left for 2 h to allow the liquid to be absorbed. Two microliter of the bacterial suspension was spot-inoculated onto the hydrated bottom film of the Petri film Select *E. coli* count plate and left for 10 min before the top film was pressed back. The inoculated Petri film Select *E. coli* count plates were incubated at 42 °C for 24 hrs as previously done (Wu et al., 2008). *E. coli* colonies appeared as round medium sized dark green to light blue-green.

#### 5.3.4 Pulsed field gel electrophoresis

Genetic relatedness of *E. coli* isolates was assessed by Pulsed Field Gel Electrophoresis (PFGE) according to a standard protocol (Ribot et al., 2006). In brief, bacteria culture in 10 ml LB broth was centrifuged at 4000 rpm at 10°C, supernatant was removed and the pellet was re-suspended and washed in Cell Suspension Buffer (CSB). Bacteria in 1% Seakem Gold agarose solution were mixed with proteinase K and incubated in buffer master mix in a water bath at 55 °C with a shaking of 70-90 rpm for 2 hrs and then washed twice in distilled water and four times in TE buffer. The plugs were held at 4°C before digestion. Plugs were cut into 2 mm blocks and transferred to preservation tubes containing 200 µl buffer mix and incubated at room temperature for 15 min. The buffer mix was then removed before

addition of *XbaI* enzyme buffer (177.5 µl MillQ water, 20 µl NE4+BSA and 2.5 µl *XbaI*). The blocks in enzyme buffer were incubated at 37°C for 2 hrs. Plugs containing *S. enterica* serovar Braenderup as well as Low Range PFGE marker (NO350S, New England Biolabs, Ipswich) were included as a reference. Separation of fragments was performed in 1% Seakem Gold agarose in 0.5xTB and the program was set at initial switch time of 2.2 sec, end switch time of 54.4 sec, run time 19 hrs, 6 Volts at an angle of 120°C. PFGE gel pictures in TIFF format were analyzed by GelCompar II software (Applied Maths, St-Martens-Latem, Belgium) using the Dice similarity coefficient and clustering by Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Band position tolerance and the optimization coefficient were both set to 1.4%. Isolates with 100% homology formed a clonal complex and were considered genetically identical.

#### 5.3.5 Comparison of cluster attributes for transmission of *E. coli*

Clusters with *E. coli* isolates showing genetic resemblance were compared with clusters without evidence of *E. coli* genetic relatedness. The status of genetic relatedness in the cluster (there is genetic relatedness or not) as the dependent variable was assessed for association with predictor variables by use of logistic regression analysis in SAS version 9. Predictor variables included presence of animal species other the cattle in the cluster, animal house roof, animal house floor, animal feeding system, application of bedding material on animal house floor, animal water source, manure collection and disposal methods, manure collection frequency, manure treatment, manure disposal distance, treatment effluent from animal house, household area and cattle and manure responsible personnel. The final model

building strategy followed a backward stepwise approach preceded by univariate analysis of all variables. The cutoff point for univariate analysis was p-value of 0.25 while for multivariate analysis the cutoff p-value was set at 0.05. The association between cattle and manure management practices between clusters was assessed by the use of Chi-square test at significance level of 0.05.

#### 5.3.6 Ethical clearance

Permission to conduct this study was granted by the Tanzania National Institute for Medical Research (NIMR) Ethical Board (certificate No. NIMR/HQ/R 8a/Vol. IX/927).

## 5.4 Results

One hundred and eighteen *E. coli* isolates that grew in presence of ampicillin and tetracycline were obtained from subcultures of 1,046 samples. Among them 40 (34%) isolates were from human stool, 50 (42%) from cattle feces, 21 (18%) from soil and seven (6%) from water samples. The ampicillin-tetracycline resistant *E. coli* isolates originated from 44 out of the total 100 clusters (44%), while the samples from the remaining clusters did not yield such resistant *E. coli*. Analysis of PFGE pattern revealed nine clonal complexes, which were designated arbitrary letters A, B, C, D, E, F, G, H and I for distinguishing purposes (Figure 1.). Inclusion of *S. enterica* serovar Braenderup in all the gels showed a band pattern reproducibility of 100% as depicted by clonal complex C. Sixteen clusters out of 44 (36%) had at least one isolate that was 100% identical to another isolate but from another source within the same cluster. Seven clusters (16%) had isolates identical by more than 95% and less than 100% (Table 1). This finding suggests that transfer of *E. coli* was a frequent

event within clusters. Clonal complexes A, D and E had five, three and six clusters respectively whose isolates from humans, cattle, soil and water shared genetic characteristics whereas clonal complexes B, F, G, F, G, H and I had two clusters each. Some clusters e.g. cluster seven, had isolates from human keeping cattle and non-cattle keeping neighbors (7H1 and 7H2), but also isolates from cattle were related to isolates from neighboring non-cattle keeping humans (cluster 6, Table 1). Isolates from water sources of non-cattle keeping households were 100% related to isolates from cattle and soil from cattle keeping households (clonal complex E, Table 1).

Logistic regression, with Hosmer and Lemeshow test  $p=1.0$ , indicated that animal house infrastructures were associated with transmission of bacteria within and between clusters. For instance, there was more bacteria transmission in clusters whose animal houses had no roof (OR=19.2, CI: 2.04-179.8). On the other hand, clusters disposing of manure within residential premises had fewer transmissions compared to households that disposed manure outside residential areas (OR=0.4, CI: 0.17-0.89) as shown in Table 2. There was an association between animal feeding system and animal water sources ( $p=0.00$ ), such that cattle under zero grazing shared water sources with humans. Manure disposal distance was associated with manure treatment method ( $p=0.005$ ). That is disposing of manure within residential premises was common in clusters that heap manure whereas direct spread of fresh manure on land was done outside residential areas.

## 5.5 Discussion

### 5.5.1 Clonal relationship between isolates

In this study *E. coli* isolates had indistinguishable band pattern within clonal complexes. Since *E. coli* is a genetically diverse organism (Tenailon et al., 2010), this must likely be due to interaction between humans, cattle, and the environment of cattle keeping and neighboring non-cattle keeping households within clusters. However, there were inter-cluster similarities in band patterns. The transmission of bacteria could be due to the type of cattle and manure management system whereby cattle defecate haphazardly during daytime communal grazing and the overnight accumulated manure in animal houses are spread around residential areas (Lupindu et al., 2012). Moreover, type of animal house infrastructure as reported in the present study was associated to bacteria transmission within and between clusters. Clusters with animal houses without roof were associated with recovery of genetically related bacteria isolates from cattle, humans, water and soil. The possible reason could be that animal houses without roof allow rain run-off water to sweep the manure from animal houses into the surrounding land and water sources and so increasing the chance of transmission. Earlier studies reported that improved animal house infrastructures such as roof, floor and drainage retained nutrient content of manure and thus improved manure quality (Lekasi et al., 2003). Combination of information from previous study (Lekasi et al., 2003) and the present study give evidence that improved animal house infrastructures can minimize both loss of nutrients from manure and risk of pathogen transmission between animals, humans and the environment.

The present study reports low magnitude of bacteria transmission within and between clusters that disposed manure within residential areas compared to clusters that disposed manure outside residential premises. However, there was significant association between manure treatment by heaping and manure disposal within residential areas while spread of fresh manure onto land was related with disposal of manure outside residential premises. It has been reported that manure treatment before disposal reduces pathogen load and survival time, and especially heaping of manure is more effective compared to other manure treatment methods (Nicholson et al., 2005; Heinonen-Tanski et al., 2006). This allows for composting which generates heat which kills pathogens. Therefore, the risk of pathogen transmission was low when treated manure was disposed within residential areas compared to disposing fresh manure to nearby surroundings.

The demonstration of clonal relationships among bacteria isolates is a common way to establish the source of infection when there are health problems to (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). Sometime, there are transmissions of bacteria to humans from animals or the environment without occurrence of disease symptoms (Silvestro et al., 2004). This study shows evidence of bacteria transmission in cattle keeping environment demonstrated by analysis of non-pathogenic bacteria as a surrogate for pathogenic ones. This choice was made because a *priori* assumption was that pathogen load would be too low to be able to make statistically valid conclusions. For example, a recent study has demonstrated that cattle kept under similar conditions in the same region of Tanzania have prevalence of *E. coli* O157:H7 of 0.9% and *Salmonella* prevalence below 0.5% (Lupindu et al., submitted). The results show that this approach can be used as a



precautionary alert to the community so that appropriate measures can be taken to prevent human or animal infection or environmental contamination

#### 5.5.2 *E. coli* isolates comparison

Bacterial isolates from humans, cattle and the environment have been compared during disease outbreaks by the use of PFGE in order to establish the possible sources of infection (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). The PFGE results from the present study give evidence that there were bacterial transmissions between humans, cattle and the environment because the band patterns of the bacteria isolates were identical. Appearance of isolates from cattle in all clonal complexes and clusters suggests that cattle could be a possible source of the bacteria to humans, water and soil. This calls for safe cattle and manure management practices so as to stop this mode of bacterial transmission which could also be used by pathogenic microorganism to cause human infection.

#### 5.5.3 *E. coli* selection criteria

Ampicillin and tetracycline are used in Tanzania as first-line and inexpensive treatment of human and animal diseases (Moyo et al., 2010). Consequently, resistance of pathogens from clinical isolates to ampicillin and tetracycline has been on a rise (Urassa et al., 2000; Moyo et al., 2010). Transmission of antimicrobial resistant *E. coli* has been reported among cattle in clusters following experimental feeding of antimicrobials containing feed (Mirzaagha et al., 2011) and between food animals and humans (Vieira et al., 2011). Hence, antimicrobial resistant *E. coli* was considered, in this study, to be possible indicators of bacteria transmission due to interaction between cattle, humans and the environment. For that purpose, *E. coli*

that share common attributes, double-drug resistance to ampicillin and tetracycline were used. Isolation of resistant *E. coli* was accomplished by the use of Petri film Select *E. coli* Count Plates (Wu et al., 2008) which directly distinguished *E. coli* from other bacteria which are  $\beta$ -glucuronidase negative and also discriminated resistant from non-resistant *E. coli*.

## **5.6 Conclusion**

Interaction between humans, animals and the environment has resulted into transmission of non-pathogenic fecal bacteria between animals, humans and the environment. The same propagation mode can lead to human or animal infection or environmental contamination by pathogens. Therefore, ways to reduce direct contact with manure and reduce pathogen load in manure and education to the community about fecal-borne pathogen should be given due weight by appropriate stakeholders so as to safeguard public, animal and environmental health.

## **5.7 Acknowledgement**

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Table 1: Clusters where identical PFGE patterns were observed in ampicillin and tetracycline resistant *E. coli* isolates from different sources.

Profile	Cluster	Isolate ID*	Source
A	6	6B2	Bovine
		6B4	Bovine
		6B6	Bovine
		6H2	Human
	7	7B2	Bovine
		7H1	Human
		7H2	Human
	8	8B1	Bovine
		8H2	Human
		8S1	Soil
9	9B3	Bovine	
30	30B1	Bovine	
B	36	36B1	Bovine
	38	38H2	Human
D	28	28H2	Human
		28S1	Soil
	4	4B1	Bovine
	3	3S1	Soil
	E	11	11B1
17		17B2	Bovine

		17S1	Soil
	18	18S1	Soil
	20	20B2	Bovine
		20B3	Bovine
	40	40W2	Water
	44	44W2	Water
	33	33H1	Human
F	9	9S1	Soil
		26B3	Bovine
G	26	26H1	Human
	26	26H2	Human
H	1	1S1	Soil
	14	14H2	Human
I	15	15H1	Human

\*isolates from humans, water and soil with odd last digit are related to cattle farms while those with even last digit are related to non-cattle keeping neighbors



Table 2: Distribution of indistinguishable PFGE patterns among variable categories and OR for the final model variables

Variable name	Class	Frequency	Identical PFGE (%)	OR	95% CI
Animal house roof	Open house	6	83	19.2	2.04-179.8
	Roofed house	110	30	1.0	
Manure disposal distance	Within residence	64	27	0.4	0.17-0.89
	Outside residence	52	40	1.0	

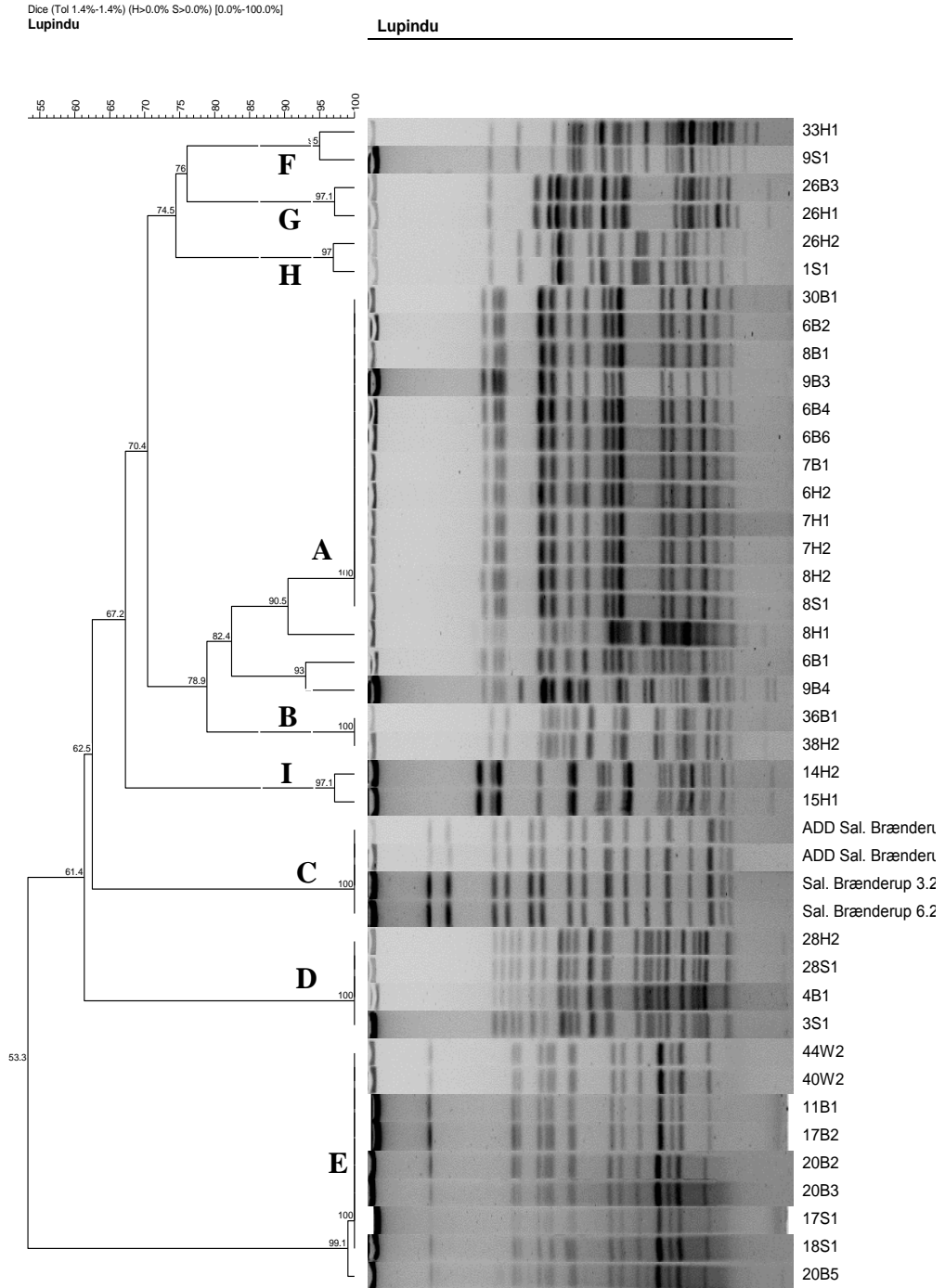


Figure 1: PFGE band pattern for ampicillin and tetracycline resistant *E. coli* isolates from humans, cattle, soil and water

## CHAPTER SIX

6.0 REVIEW OF THE EPIDEMIOLOGY OF SHIGA TOXIN-PRODUCING  
*ESCHERICHIA COLI* O157:H7 IN AFRICA (Manuscript V)

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## 6.1 Abstract

*Escherichia coli* O157:H7 is responsible for intestinal and extra-intestinal disease syndromes in human. Infected animals, food products and contaminated environment serve as sources of human infection. Isolation of the pathogen from animals, vehicles and clinical samples are reported from all continents even though much work has been conducted in developed countries. Literature shows that about a quarter of African countries have reported recovery of *E. coli* O157:H7 from either humans, animals, food products or the environment. Most *E. coli* isolations from human patients do not occur concomitantly with reports of isolation of the pathogen from other sources such as animals, water or food products. The present review describes the current epidemiological status of the pathogen on the aspects of source, transmission, diagnosis, treatment and control strategies, disease burden and the challenges presented by the new emerging human prone group. The aim is to give an insight into the situation and bring to the attention of different stakeholders on the public health threat of the pathogen for possible formulation and implementation of multi-sectoral control strategies.

Keywords: *E. coli* O157, STEC, EHEC, HUS, Africa

## 6.2 Introduction

*Escherichia coli* strains that cause diarrhea in human are either enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) or enterohemorrhagic *E. coli* (EHEC) (Nataro and Kaper, 1998). One of the EHEC strains associated with diarrhea, bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS) also described as Shiga toxin-producing *Escherichia coli* (STEC), is *E. coli* O157:H7 (Riley et al., 1983). Once described as a rare serotype causing human infection (Wells et al., 1983), *E. coli* O157:H7, is now widespread in food products (Chahed et al., 2006; Kang'ethe et al., 2007; Beneduce et al., 2008) and the environment (Müller et al., 2001; Howie et al., 2003). This widespread nature and other biological characteristics such as low infective dose (Hancock et al., 1994; Strachan et al., 2001; Howie et al., 2003; Caprioli et al., 2005), ability to express different virulence factors (Caprioli et al., 2005), long time survival in the environment (Avery et al., 2005) and the difficulty in treatment (Germani et al., 1997), make *E. coli* O157:H7 an enteropathogen of major concern worldwide. This paper reviews the epidemiology of *E. coli* O157:H7 in Africa, gathering information about (1) distribution, (2) disease picture, (3) pathogenicity, (4) isolation and characterization, (5) treatment (6) disease burden and (7) relation with other diseases such as HIV/ AIDS so that the veterinary and medical sectors as well as the community are made aware of this health problem in Africa. The temporal spectrum of this review starts in 1983, when the pathogen was reported for the first time to cause disease in human, to the year 2012. Most of the reviewed work comes from

Africa but we have included a few others from outside Africa for a broader view of the subject.

### **6.3 The global burden of STEC O157:H7 infection**

The burden of STEC O157 infection is immense as shown by quantified data from developed countries. An estimate of 73,480 cases of STEC O157 illness has been reported to occur annually in the United States of America with case mortality case tallying to 0.0083. Apart from other sources, 85% of these cases were food-borne (Mead et al., 1999). For the year 2000 to 2001, an average of 2173 STEC cases were reported with a 0.4-2.0 rate per 1000 population (Thomas et al., 2006). In the Netherlands the incidence of STEC O157 infection from 1990 to 2000 was 1250 cases per year, out of which 20 were HUS cases and 2.5 fatalities per year (Havelaar et al., 2004). Lack of data about human morbidities and mortalities at regional and country levels in Africa blur the magnitude of the problem but isolation record of STEC O157:H7 from different animal species, foodstuff and the environment from all over Africa suggest that humans are at risk and probably many cases pass unnoticed.

### **6.4 Reports of *E. coli* O157:H7 Occurrence in Africa**

*Escherichia coli* O157:H7 has been reported from all over Africa (East, Central, South, North and West Africa) from human, animals, food products or the environment. The first case of *E. coli* O157:H7 human infection was reported back in Johannesburg, South Africa in 1990 (Browning et al., 1990).

### **Central Africa**

In Central Africa *E. coli* O157:H7 caused hemorrhagic colitis outbreak in Bangui (Germani et al., 1997). Moreover, the first *E. coli* O157:H7 isolation from humans was reported in 1998 following an outbreak of bloody diarrhea in Cameroon (Cunin et al., 1999). Reports from other Central African countries such as Chad, Democratic Republic of Congo, Equatorial Guinea, Gabon, Sao Tome and Principe, Republic of Congo and Angola were not available.

### **East Africa**

In East Africa *Escherichia coli* O157:H7 has been reported in Tanzania, Kenya and Ethiopia. A prevalence of more than 7% *Escherichia coli* O157:H7 in human patients with diarrhea was reported in Morogoro, Tanzania (Raji et al., 2008). In Kenya, *E. coli* O157:H7 was isolated from a 2 year old boy with hemorrhagic colitis (Sang et al., 1996) and later milk and cattle feces were reported positive for *E. coli* O157:H7 in the same country (Kang'ethe et al., 2007). *E. coli* O157:H7 has been isolated from beef, mutton and chevon in Ethiopia at a prevalence of 8%, 2.5% and 2% respectively (Hiko et al., 2008). This strain has also been recovered in the same country from goat and sheep feces (4.7%), skin swabs (8.7%), carcass before washing (8.1%) and carcass after washing (8.7%) and water samples (4.2%) (Mersha et al., 2010). No data were available about *E. coli* O157:H7 from Uganda, Somalia, Sudan, Eritrea, Djibouti, Rwanda and Burundi. Nevertheless, this does not rule out possibility of presence of *E. coli* O157:H7 in these countries.

### **North Africa**

In North Africa which includes Algeria, Morocco, Tunisia, Libya and Egypt reports on *E. coli* O157:H7 occurrence are available in all countries except Libya. A study in Algeria reported a prevalence of 7% pathogenic *E. coli* O157:H7 from bovine carcasses (Chahed et al., 2006). In Morocco, a prevalence *E. coli* O157:H7 of 9.1% from dairy products and 11.1% meat marketed in Rabat has been reported (Benkerroum et al., 2004). Later in the same country, *E. coli* O157:H7 was isolated from raw meat products at a proportion of 9% (Beneduce et al., 2008) and in 2011 a 1.9% prevalence of *E. coli* O157:H7 from shellfish and marine environment of Morocco was reported (Bennani et al., 2011). In Tunisia, 3.4% of isolates from human stool samples possessed Shiga-Toxin producing genes of *E. coli*, including one strain of *E. coli* O157:H7 (Al-Gallas et al., 2006). Isolation of *E. coli* O157:H7 from different sources has also been documented from Egypt. For instance, a survey in middle Egypt revealed prevalence of *E. coli* O157:H7 of 6% from beef samples, 4% from chicken samples, 4% from lamb samples and 6% obtained from milk samples in slaughterhouses, supermarkets and farmers' homes (Abdul-Raouf et al., 1996).

### **West Africa**

In West African region, much of the work on *E. coli* O157:H7 has been reported in Nigeria. In Lagos a prevalence of *E. coli* O157:H7 of 6% from patients with diarrhea has been documented (Olorunshola et al., 2000). In another city of Ibadan, *E. coli* O157:H7 from feces of cattle, sheep, goat and pigs and also from beef, goat-meat and pork at a rate of 5% was isolated (Ojo et al., 2010). In Zaria, Nigeria, *E. coli*



O157:H7 has been isolated from diarrheal stool of children under the age of 5 years at the rate of 5.4% and from surface water at rate of 2.2% (Chigor et al., 2010). The *E. coli* O157:H7 isolation in Nigeria provides evidence of occurrence of the pathogen in human, animals, meat and environment (water). A study in the Coastal Savannah zone of Ghana did not report isolation of *E. coli* O157H7 in raw milk and milk products (Addo et al., 2011), but this does not guarantee absence of the pathogen. Information on recovery of *E. coli* O157:H7 from other western African countries including Mali, Niger, Guinea, Ivory Coast, Togo, Benin, Guinea Bissau, Sierra Leone, Liberia, Mauritania, Cape Verde and Burkina Faso were not accessed. But given similar environment, there is high chance that this pathogenic *E. coli* strain exists in these countries.

### **Southern Africa**

The South African region is comprised of Zambia, Malawi, Mozambique, Zimbabwe, Botswana, Namibia, Swaziland, Lesotho and South Africa. In South Africa a prevalence of *E. coli* O157:H7 of 10.3% from vegetable samples in Eastern Cape Province has been documented (Abong'o and Momba, 2008). Meat and meat products from the same location carried *E. coli* O157:H7 at a rate of 2.8% (Abong'o and Momba, 2009). Further studies reported *E. coli* O157:H7 prevalence of 56.5% and 43.5% from stool of confirmed and non-confirmed HIV/AIDS patients in Eastern Cape Province (Abong'o et al., 2008). The *E. coli* O157:H7 isolates from meat products (7.8%), water (8.6%), vegetables (10.3%), confirmed HIV/AIDS patients (56.5%) and non-confirmed HIV/AIDS patients (43.5%) were genetically related (Abong'o and Momba, 2008). In the neighboring country of Botswana, the

prevalence of *E. coli* O157:H7 in meat cubes, minced meat and fresh sausages in Gaborone were reported to be 5.22%, 3.76% and 2.26% respectively (Magwira et al., 2005). This finding from beef product outlet put consumers at risk of infection with *E. coli* O157:H7. Home cooked food samples (maize flour porridge, fish, vegetables, beans) investigated for pathogenic bacteria, were found to be contaminated with pathogenic *E. coli* O157:H7 at a rate of 8% in Lungwena – Malawi (Taulo et al., 2008). In Mozambique *E. coli* O157:H7 is reported to be one of the causes of diarrhea in children at a proportion of 1.9% (Mandomando et al., 2007). Moreover, this review captured a report of *E. coli* O157:H7 causing dysentery in HIV patients in Zimbabwe at a rate of 8% (Gwavava et al., 2001).

Therefore, reports on isolation of pathogenic *E. coli* O157:H7 from all regions of the African continent (East, West, South, North and Central) show that the pathogen is present all over Africa. A total of 15 countries have reported recovery of pathogenic *E. coli* O157:H7 either from human, animals, food products or the environment. Out of 28 reviewed cases, 10 (35.7%) come from human patients and the remaining 18 isolations belong to food stuffs (8), cattle (4), water (2) others ( 2 sheep and goats, 1 vegetable and 1 shell fish and environment) (Table 1).

Table 1: Sources of *E. coli* O157:H7 isolation across African continent

Country	Source	Identification method *	Author
Algeria	Cattle	SMAC, PCR, DNA hybridization	Chahed et al. 2006

Botswana	Food	IMS, SMAC and anti O157 antisera	Magwira et al. 2005
Cameroon	human	SMAC, anti O157 antisera, and VCA	Cunin et al. 1999
Central African Republic	human	PCR	Germani et al. 1997
Egypt	Cattle, fish, water, environment	SMAC, anti O157 antisera and VCA	Tuyet et al. 2006
Ethiopia	food	SMAC and anti O157 antisera	Abdul-Raouf et al. 1995
Kenya	Food	SMAC, Anti O157 antisera	Hiko et al. 2008
	Water, sheep, goats	SMAC, anti O157 antisera and PCR	Mersha et al. 2010
Malawi	human	PCR	Sang et al. 1996
	Cattle, food	SMAC, anti O157 antisera and PCR	Kang'ethe et al. 2007
Morocco	Food	SMAC, anti O157 antisera	Taulo et al. 2008
Morocco	food	IMS, SMAC, anti O157 antisera and PCR	Benkerroum et al. 2004
	Shellfish,	SMAC, anti O157	Beneduce et al. 2008 Bennani et al. 2011

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	environment	antisera and PCR	
Mozambique	Human	PCR	Mandomando et al. 2007
Nigeria	human	SMAC, anti O157 antisera. VCA	Olorunshola et al. 2000
	Cattle, food, sheep, goat, pig	SMAC and anti O157 antisera	Ojo et al. 2010
	Human, water	SMAC, anti O157 antisera	Chigor et al. 2010
South Africa	Human, vegetable	IMS, SMAC and PCR	Abong'o et al. 2008
	Food	IMS, SMAC and PCR	Abong'o and Momba 2009
Tanzania	human	SMAC, IMS, anti O157 antisera and PCR	Raji et al. 2008
Tunisia	human	SMAC, anti O157 antisera, PCR and VCA	Al-Gallas et al. 2006
Zimbabwe	Human	SMAC	Gwavava et al. 2001

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\*SMAC = sorbitol MacConkey agar, IMS= immunomagnetic separation, VCA=

Vero cell assay

## 6.5 Transmission of *E. coli* O157:H7

*Escherichia coli* O157:H7 is an enteric pathogen that is transmitted to human through ingestion of contaminated food or hands to mouth (Tuttle et al., 1999; Howie et al., 2003). Person to person contact can lead to transmission of the pathogen through oral-fecal route (Caprioli et al., 2005). The infectious dose that has caused disease symptoms in human has been reported to be as low as 4 to 24 organisms (Strachan et al., 2001; Howie et al., 2003). Ruminants are said to be reservoirs whereby cattle are regarded principal sources (Kaddu-Mulindwa et al., 2001; Heuvelink et al., 2007; Kang'ethe et al., 2007; Ateba and Bezuidenhout, 2008; Cobbaut et al., 2009; Ateba and Mbewe, 2011). However other ruminant species such as goats and sheep (Mersha et al., 2010; Ojo et al., 2010), buffaloes (Dorn and Angrick, 1991), serve as a source of pathogenic *E. coli* O157:H7, except for camels (El-Sayed et al., 2008). Non-ruminant animals such as pigs (Ateba and Bezuidenhout, 2008; Ojo et al., 2010; Ateba and Mbewe, 2011) and pigeons (Shere et al., 1998) are also reported to carry the *E. coli* O157:H7 strain. Fish in contaminated water have been reported to harbor *E. coli* O157:H7 (Tuyet et al., 2006). A single dose of 100 CFU of *E. coli* O157:H7 is sufficient to infect cattle (Hancock et al., 1997) while sheep has been reported to be infected by a single oral dose of  $10^5$  CFU of *E. coli* O157:H7 (Kudva et al., 1998). These doses can be acquired by ingestion of as little as 0.1g of manure containing  $10^6$  CFU/g (Kudva et al., 1998). Shedding of *E. coli* O157:H7 in cattle is intermittent (Besser et al., 1997; Shere et al., 1998). The duration of *E. coli* O157:H7 shedding by cattle is less than a month and the peaks occur during the months of summer in countries North of Equator (Besser et al., 1997; Hutchison et al., 2005). Calves around weaning are

reported to shed more bacteria than other age groups (Laegreid et al., 1999; Hutchison et al., 2005). These findings suggest that having negative results at a particular point in time does not necessarily mean absence of *E. coli* O157:H7. Moreover, the reported prevalence of *E. coli* O157:H7 may be lower or higher than the real situation depending on the composition cattle by age in the study.

Accidental ingestion of *E. coli* O157:H7 following contact with infected animals or contaminated environment has led to human infection (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). But food products such as beef (Abdul-Raouf et al., 1996; Benkerroum et al., 2004; Magwira et al., 2005; Chahed et al., 2006; Beneduce et al., 2008; Hiko et al., 2008) seem to be easily contaminated and become a major source of *E. coli* O157:H7 infection. However, *E. coli* O157:H7 has been isolated from chevon and mutton at abattoir or in retail outlets (Abdul-Raouf et al., 1996; Hiko et al., 2008; Mersha et al., 2010). Other food products reported to harbor *E. coli* O157:H7 are milk and chicken from supermarkets and farmer's homes (Abdul-Raouf et al., 1996; Benkerroum et al., 2004). Marine environmental contamination has also posed a risk because of isolation of *E. coli* O157:H7 from shellfish in Morocco (Bennani et al., 2011). Convenient foods under poor preparation or handling have also been reported to play a role in propagation of this pathogen (Tuttle et al., 1999). Moreover inanimate objects such as soil (Howie et al., 2003), water (Tuyet et al., 2006; Chigor et al., 2010) marine sediments (Bennani et al., 2011) and manure (Hutchison et al., 2005) act as a source of *E. coli* O157:H7. The risk is potentiated by the ability of the pathogen to survive harsh conditions such as low pH of dairy products (Tsegaye and Ashenafi, 2005; Dlamini and Buys, 2009), or in manure more

than four months (Kudva et al., 1998). Generally, the risk factors for *E. coli* O157:H7 infections remain to be contact with animals and their environment and personal hygiene such as not washing hands after handling animals or prior to eating (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). These findings and reports call for hygiene observance after contact with animals, suspected environment or during preparation of foods.

### **6.6 Disease caused by *E. coli* O157:H7**

*Escherichia coli* O157:H7 has been reported to cause disease symptoms in human. Disease symptoms may take different forms such as diarrhea (Raji et al., 2008), hemorrhagic colitis (Sang et al., 1996; Germani et al., 1997) or hemolytic uremic syndrome (Germani et al., 1997). Hemolytic uremic syndrome, which is characterized by thrombocytopenia, hemolytic anemia and nephropathy, may come as a complication of *E. coli* O157:H7 infection following prolonged illness or sometimes disease management such as the use of antibiotics (Wong et al., 2000). However, some humans do not show signs of disease despite infection and these are asymptomatic carriers (Silvestro et al., 2004; Al-Gallas et al., 2006).

Disease syndromes by *E. coli* O157:H7 have been reported to take the form of an epidemic (Germani et al., 1997; Cunin et al., 1999) whereby the 1992 outbreak in Swaziland and South Africa are reported to be the largest in Africa (Effler et al., 2001). However, sporadic forms of the disease have posed a threat to public health as well (Sang et al., 1996).

## 6.7 Pathogenicity of *E. coli* O157:H7 infection

Enterohemorrhagic *E. coli* O157:H7 possesses different virulence factors that are important in pathogenicity. The major virulence factor is intimin that is coded by attaching and effacing (*eae*) gene (Jerse et al., 1990). Intimin is reported to facilitate attachment of bacteria to intestinal epithelia during colonization resulting into production of lesions and diarrhea (Moon et al., 1983; McDaniel et al., 1995; Gallien, 2003a). This virulence factor is also possessed by enteropathogenic *E. coli* (EPEC) (China et al., 1999). Another virulence factors for *E. coli* O157:H7 are the shiga toxins. Two forms of the toxins, *stx1* and *stx2* encoded by *stx1* and *stx2* genes (Gallien, 2003a) are reported to be responsible for hemorrhagic uremic syndrome (HUS) (Paton and Paton, 1998). Shiga toxins, which are protein molecules, bind to eukaryotic surface cells and inhibit protein synthesis with a consequence of death of host cells (Sandvig, 2001). Enterohaemolysin is another virulence factor for *E. coli* O157:H7. This protein toxin damages cell membrane of erythrocytes and is used as a surrogate tool in detection of shiga producing *E. coli* (Schmidt et al., 1995; Jürgens et al., 2002; El Sayed Zaki and El-Adrosy, 2007). Although enterohaemolysin activity can easily be visualized on blood agar cultures, confirmation is usually achieved by PCR amplification of *ehxA* gene (Gallien, 2003b; El Sayed Zaki and El-Adrosy, 2007). Some other *E. coli* strains such as O26, O103, O111, O118, O128, O121, O45 and O145 can produce disease syndrome and have been reported to be enterohaemolysin positive and produce shiga toxins (Schmidt et al., 1995; Gyles et al., 1998; El Sayed Zaki and El-Adrosy, 2007). The synergic effects of these virulent factors make *E. coli* O157:H7 a potential pathogen to humans



## **6.8 Detection of *E. coli* O157:H7**

*Escherichia coli* O157:7 can be isolated by use of Sorbitol MacConkey agar whereby most *E. coli* O157:H7 are distinguished from other strains by their inability to ferment sorbitol. Direct inoculation of sample on sorbitol MacConkey agar plating has been employed but has proved less sensitive compared to immunomagnetic separation (Chapman and Siddons, 1996; Cubbon et al., 1996). Either of the options is accomplished by agglutination test using antibodies against somatic antigen O157. However, polymerase chain reaction (PCR) for the detection of verocytotoxins in *E. coli* O157:H7 remains a gold standard detection method (Cubbon et al., 1996). Detection of the bacteria or toxins may take more than 24 hrs (Gould, 2012). However, accurate and quick detection method of *E. coli* O157:H7 or verocytotoxins is desirable so as to enable rapid attendance of patients especially in outbreaks.

## **6.9 Treatment of STEC O157:H7 infection**

Infections with shiga toxins-producing bacteria such as *Shigella dysenteriae* type I and STEC are controlled by the use of antibiotic and supportive therapies (Germani et al., 1997; Bennish et al., 2006). However, in complicated forms of infection, like with HUS, antibiotics are not effective (Germani et al., 1997; Bennish et al., 2006). Administration of antibiotics to patients infected with STEC O157:H7 is reported to increase release of shiga toxins and thus increasing the risk of developing HUS (Germani et al., 1997; Wong et al., 2000). This is thought to be due to increased release of toxins following death of STEC (Wong et al., 2000). The case is different, however, in *Shigella dysenteriae* type I infection where early antimicrobial therapy lowers the risk of developing HUS (Bennish et al., 2006). Therefore it is important to

establish the etiology of an enteric disease before administration of antibiotics because it may worsen the prognosis in case of STEC infection. This demand presents a challenge in developing countries where diagnostic services do not match the requirements and antibiotics are haphazardly used (Hounsa et al., 2010; Vento and Cainelli, 2010).

### **6.10 Control of STEC O157:H7 infection**

Research on vaccination of reservoirs as an effort to reduce bacteria shedding has shown signs of success (Potter et al., 2004) but the practicability of this approach is questionable because of the use of transgenic tobacco plant cells (Caprioli et al., 2005). Some substances such as essential oils from *Cinnamomum zeylanicum* have shown bacteriocidal activities (Senhaji et al., 2007). Nevertheless, the above efforts plus dietary manipulations are not promising strategies. Thus, hygienic management of animals and food products remain better options in control of STEC transmission. Moreover, we suggest institution of an inter-sectoral cooperation between veterinary (where the main reservoir, cattle, belong) and medical profession (where patients are cared for). A platform for exchange of information and strategies can help in control emerging and spread of the pathogen.

### **6.11 Emerging human prone group**

Shiga toxin producing *E. coli* infect all sexes and ages, but many reported cases involve young and elderly people (Sang et al., 1996; Mandomando et al., 2007). Of recent, the susceptibility spectrum has widened to include a section of people living with HIV/ AIDS (Gwavava et al., 2001; Abong'o et al., 2008). This poses a new

challenge because some forms of STEC infections e.g. HUS are aggravated by the use of antibiotics which are essential in HIV/AIDS patients to combat other infections due to opportunistic microorganisms. However, antibiotics such as ciprofloxacin, meropenem, fosfomycin, chloramphenicol azithromycin, and rifaximin did not induce production or release of shiga toxins when used in treatment of STEC O104:H4 infection (Bielaszewska et al., 2012; Corogeanu et al., 2012). More research on these antibiotics is desired to check for possibility of using them to patients with HIV/AIDS.

#### **6.12 Is *E. coli* O157:H7 zoonotic?**

Adult cattle do not go down with disease syndromes due to lack of vascular receptors for shiga toxins produced by *E. coli* O157:H7 (Pruimboom-Brees et al., 2000) but experimental infection in has resulted into fatal iliocolitis in calve (Dean-Nystrom et al., 1998) and diarrhea and neurological signs in piglets (Dean-Nystrom et al., 2000). Other animal species such as sheep, goat, water buffalo, deer, horse, rabbits, pigs and pigeons can be infected by STEC O157:H7 but do not show disease signs (Caprioli et al., 2005). By definition, zoonoses are diseases or infections which are naturally transmitted between vertebrate animals and man (WHO, 1959). Therefore, *E. coli* O157:H7 is a zoonotic pathogen because it can naturally infect both vertebrate animals and humans with the latter group showing disease syndromes.

#### **6.13 Conclusion**

Isolation of *E. coli* O157:H7 from animals and food products reported from almost all over Africa suggests a high risk of human infection. Lack of proper laboratory

facilities especially in rural settings of Africa interferes with making definitive diagnosis and hence patients are not treated appropriately. As such, antibiotics prescription to patients with gastroenteritis can be fatal especially in case of *E. coli* O157:H7 infection. Additionally, difficulty in managing *E. coli* O157:H7 infection cases and time consuming diagnostic procedures call for preventive approaches rather than curative measures. Proper cattle and manure handling practices as well as public awareness on the epidemiology of the pathogen should be instituted. Vehicles of *E. coli* O157:H7 transmission such as food products and water should be made free of the pathogen so as to prevent health implications due to *E. coli* O157:H7 infection

#### **6.14 Acknowledgement**

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## 7.0 GENERAL DISCUSSION

The present study reports isolation of zoonotic bacteria from cattle and water sources and that diarrheagenic *E. coli* were not isolated from human subjects, both cattle keepers and non-cattle keepers. The reason for missing the diarrheagenic *E. coli* from humans may be due to taking stool samples from healthy human subjects. The expectation was that healthy humans could carry diarrheagenic *E. coli* such as strain O157:H7 without clinical manifestation since carrier state has been reported (Silvestro et al., 2004). Involving diseased human subjects in the study could have given the diarrheagenic *E. coli* infection status of the population e.g. by taking stool samples from diarrheagenic patients in hospitals as previously done (Raji et al., 2008) but their contact with cattle and or manure could not be guaranteed. Hence, the influence of cattle and or manure handling on human infection with zoonotic bacteria could not be easily assessed. Absence of infected humans with zoonotic bacteria from both cattle and non-cattle keeping households blurred quantification of risk of zoonotic bacterial infection. Nevertheless, transmission of bacteria between cattle, humans and the external environment was assessed using commensal *E. coli* by using antimicrobial resistance and PFGE procedures

The use of PFGE to trace sources of infection in outbreaks is common whereby the DNA band patterns of isolates from patients and suspected sources of infection are compared and judged identical when the band pattern resemblance is 100% (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). However, combination of this method with other screening criteria adds inference certainty regarding genetic relatedness between bacteria isolates. In the present study, double antimicrobial

resistance to ampicillin and tetracycline of *E. coli* isolates preceded PFGE fingerprinting, thus giving strong evidence of genetic relatedness among isolates from cattle, humans, soil and water.

Assessment of infection status in human subjects from cattle keeping households focused on household members who frequently handled cattle and or manure. However, manure in the study area was disposed of haphazardly in the surrounding land such that soil and water were contaminated. This cattle and manure management practice suggests that children are at risk of infection in the community due to their playful behavior that lead them to frequent contact with soil and water.

Gastroenteritis outbreaks in children due to *E. coli* O157:H7 following farm visits have been reported in USA (Crump et al., 2002; Howie et al., 2003; Smith et al., 2004). Therefore, children in cattle keeping environment form another group at risk of manure-borne pathogen infection.

Animal manure harbor pathogens such as *Campylobacter jejuni*, *Listeria monocytogens*, *Guardia* spp., *Cryptosporidium* spp., diarrheagenic *E. coli* and *Salmonella* spp. (Hutchison et al. 2004; Klein et al. 2010). In addition, zoonoses such as Tuberculosis and Brucellosis can result from animal contact or consumption of poorly processed animal products (Marcotty et al. 2009). Apart from manure, other animal related materials such as meat, milk, hides and skins should be considered as potential sources of zoonotic pathogens. Such materials were not checked for presence of a diverse spectrum of disease causing organisms in the present study. Therefore, failure to recover diarrheagenic *E. coli* or zoonotic *Salmonella* spp. in

humans does not infer that the population in close contact with cattle in urban and peri-urban livestock farming areas is not at risk, given the DNA band pattern resemblance by PFGE.

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## 8.0 GENERAL CONCLUSION

Recovery of pathogenic *E. coli* such as VTEC strains of pathotypes O157:H7, O113:H21 and O+:H16, EPEC strains (O142:H34) and untypeable A/EEC from fresh cattle feces confirms that cattle are reservoirs of pathogens that can infect humans. The EPEC strains were recovered from cattle and water hence giving an indication that there was contamination of water by cattle manure.

The bacteria isolated in this study were proven virulent since they produced toxins that had cytopathic effect to live cells. Members of households keeping cattle in the study area are at risk of infection because they are in direct contact with cattle and or manure. There is high chance of environment (soil and water) contamination since fresh manure is spread haphazardly into the surrounding land. The danger of cattle or manure-borne microorganisms to infect humans extends beyond cattle keepers to their non-cattle keeping neighbors. The present study has realized this concept by showing evidence of bacterial transmission between cattle humans and the environment. Therefore, there is a need for formulation and enforcement of manure management guidelines to make the urban and peri-urban livestock farming safe. Additionally, processing of manure to turn it into a sellable commodity is desired so as to encourage safe manure disposal.

Isolation of *S. amager* from human stool in the study area shows that the strain that was once a public health threat is still present in the community. However, its recovery from asymptomatic human subjects brings up more questions especially on

its virulence. Further research about current epidemiological status of *S. amager* is required.

The present study was conducted in urban and peri-urban areas of Morogoro Tanzania, where animals and humans live in close proximity. The results, however, can apply to areas of similar set up in developing countries where there is direct contact between humans, cattle and manure.

## 9.0 PERSPECTIVES AND FUTURE RESEARCH

The research addresses the issue of cattle and manure management relative to risk of zoonotic pathogen infection to humans and cattle. However, its cross-sectional nature gives the outcome at a specific point in time. A longitudinal study could provide better understanding on pathogen establishment in the host and possible shedding trend of the pathogens.

The present study checked the infection status of healthy human subjects who are in contact with cattle and or manure in urban and peri-urban areas of Morogoro. In order to get the health status of diarrheagenic humans, there is a need to check for potential pathogens from diarrhea patients in health care centers. Contact of patient with cattle and or manure can be established to a reasonable level if the sample size is large.

The present study focused on manure-borne pathogenic *E. coli* and Salmonella strains that can infect humans and/ or contaminate the environment. Other manure-borne zoonotic pathogens such as *Campylobacter jejuni*, *Listeria monocytogens*, *Giardia* spp. and *cryptosporidium* spp. and milk and meat-borne pathogens e.g. *Mycobacterium* and, *Brucella* spp. were not studied. Research on pathogens other than pathogenic *E. coli* and salmonella from cattle and other livestock such as pigs and poultry could give useful information on human infection risks associated with handling livestock and/ or manure.

Manure in the study area is regarded by some cattle keepers as waste by-product. Research into manure management practices that maintain nutritive value, reduce pathogen load and make it convenient to handle is desired. Prospective research should turn manure into a commodity that is convenient to handle, transport and apply.



## 10.0 APPENDIX

**10.1 Appendix 1: Questionnaire for cattle keeping households**

Title: Animals and Animal Waste Management in Urban and Peri-Urban Farming  
in Tanzania.

## INTRODUCTION

Athumani M. Lupindu, a PhD student of SUA is interested to know the animal waste management practices in urban and peri-urban areas of Morogoro, including the whole chain of activities, from collection to disposal. He is also trying to quantify bacterial enteropathogen transmission risk, if any, among the animals, human and the environment. Here is a questionnaire that can take approximately one hour to complete. It contains 39 questions focusing on different aspects. The information obtained from this interview will be handled confidentially. Results of this research will be used to write a PhD thesis and make publications. Moreover, recommendations of this study will be shared with farmers and policy makers so that the current animal waste management practices are improved for the betterment of all stakeholders. Do you have any question?

Questionnaire number .....

Date of interview: ...../...../..... (dd/ mm/ yy)

Name of interviewer .....

Designation of interviewer .....

## QUESTIONS

• **Farmer’s particulars**

1. What is the name of the farm owner? .....
2. What is the name of the village/ street? .....
3. What is the name of the ward? .....
4. What is the name of Division? .....
5. What is the name of the district? .....
6. What is your telephone number .....

• **Labor division ( household members and their responsibilities)**

7. How many members does this household have? .....

Name	occupation	Daily responsibilities
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

• **Herd characteristics and management** (size, composition, housing, feeding, presence of other species, water sources, diseases and disease management etc.)

8. What animal species do you keep?  
.....  
.....
9. How many cattle do you keep? .....

Cows ..... bulls ..... male calves ..... Female calves .....

10. Animal identification

<u>Number</u>	<u>Name/ description</u>
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

11. Where are the cattle kept?

- a) Roofed house
- b) Open boma

12. What is the animal house floor made of?

- a) Concrete
- b) Soil

13. What are the cattle feeding systems?

- a) Zero grazing
- b) Semi-intensive system

14. What is the source of water for the cattle?

- a) Stream/ river
- b) Borehole
- c) Tap water
- d) Pond
- e) Others

Specify .....

15. What is the source of water for human use?

- a) Stream/ river
- b) Borehole
- c) Tap water
- d) Pond
- e) Others

Specify .....

16. When did you treat your cattle for the last time?

.....

a) What was the disease treated? .....

b) What drug did you use to treat the disease?

.....

- **Animal waste handling** (collection [means, frequency, form], storage, disposal [means, distance] etc., farm area)

17. What is the daily activity schedule?

a) Milking → Animal house cleaning → Feeding → Milking → Self cleaning

b) Milking → Feeding → Animal house cleaning → Milking → Self cleaning

c) Milking → Self-cleaning → Feeding → Animal house cleaning → Milking

d) Milking → Animal house cleaning → Self-cleaning → Feeding →Milking

e) Milking → Feeding → Animal house cleaning → Self-cleaning →

Milking

f) Other schedule

Specify .....

18. How do you use animal waste?

g) As fertilizer

h) Discard

i) Any other use

Specify .....

19. How do you collect animal waste?

a) Hand picking

b) Spade collection

c) Water splashing

d) Do not collect

20. How often do you collect animal waste?

a) Once a day

b) More than once a day

c) Never collect

d) Other procedure

Specify .....

21. How do you carry animal waste from animal house?

a) Using wheel barrel/ bucket

b) Plastic bags

c) Bare hands

d) Spade

e) Other method

Specify .....

22. How do you treat animal waste after collection?

a) Spread to crop field directly

b) Store in a hip and spread to the field

c) Store in a hip and give away to others/ sell

23. Where do you dispose animal waste?

a) Within the homestead

b) Out of homestead premises

24. How far from living house do you store/dispose animal waste?

a) Within 10 meters from homestead

b) More than 10 meters from the homestead

25. Do have biogas digester pit?

a) Yes

b) No

26. What do you put on feet during animal waste handling

a) Gum boots

b) Ordinary shoes

c) Nothing

27. What do you put on during animal waste handling?

a) Special animal waste handling clothing

b) Ordinary clothing

28. How do you treat effluent from animal house?

- a) Direct effluent to lagoon
- b) Spread on soil

29. What is the approximate household area? .....

30. How do you wash yourself after animal waste handling?

- a) Wash hands
- b) Wash the whole body
- c) Do not wash

Why? .....  
.....

• Household history and knowledge on gastroenteritis

31. Do you suffer from gastroenteritis?

- a) Yes
- b) No

32. If no, when did you have gastroenteritis for the last time?

.....

33. Is there any household member with gastroenteritis?

- a) Yes
- b) No

34. If no, when did any household member experience gastroenteritis?

.....

• Knowledge on risk of enteropathogen transmission

35. What do you do when one member of the household has gastroenteritis?

.....  
.....

36. How can someone get infected with enteropathogens?

.....  
 .....

37. Have you ever heard of gastroenteritis due to animal waste handling?

a) Yes

b) No

- **Crow access to household premises**

38. Do you see crows coming to the household premises?

a) Yes

b) No

- **General question**

39. What problems do you face with regard to animal waste management?

.....

That was the last question of our conversation. Thank you for your answers and time. I will visit you again for taking samples from the animals, environment and personnel involved in animal waste handling. Analysis of facts obtained from your farm will be done together with the information from other farms participating in this study. The outcomes of the study will be shared among participants whenever available for the purpose of improving animal, public and environmental health. Do you have any comment or question about the interview and our conversation?



## 10.2 Appendix 2: Questionnaire for non-cattle keeping households

Title: Animals and Animal Waste Management in Urban and Peri-Urban Farming  
in Tanzania

### INTRODUCTION

Athumani M. Lupindu, a PhD student of SUA is interested to know if animal keeping and specifically animal waste handling can lead to bacterial enteropathogen transmission to human and environment. This can be achieved by comparison of the data from livestock keeping and non-livestock keeping households. Here is a questionnaire for non-livestock keepers that can take one hour to complete. It contains 24 questions focusing on different aspects. The information obtained from this interview will be handled confidentially. Results of this research will be used to write a PhD thesis and make publications. Moreover, recommendations of this study will be shared with livestock keepers, non-livestock keepers and policy makers so that the current animal waste management practices are improved for the betterment of all stakeholders. Do you have any question?

Questionnaire number .....

Date of interview: ...../...../..... (dd/ mm/ yy)

Name of interviewer .....

Designation of interviewer .....

### QUESTIONS

- **Participant's particulars**

1. What is the name of the household leader? .....

- 2. What is the name of the village/ street? .....
- 3. What is the name of the ward? .....
- 4. What is the name of Division? .....
- 5. What is the name of the district? .....
- 6. What is your telephone number? .....

• **Labor division ( household members and their responsibilities)**

7. How many members does this household has? .....

Name	Occupation	Daily responsibilities
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

• **Livestock keeping history**

8. Has the household ever kept livestock (cattle, goat or sheep)?

- a) Yes
- b) No

9. If yes, when was it? .....

10. If no, why?

- a) Lack of financial capital
- b) Shortage of land
- c) Lack of time

d) Other reasons

Mention .....

11. What other animal species (other than cattle, goat or sheep) do you keep?

.....

• **Water source**

12. What is the source of water for household use?

f) Stream/ river

g) Borehole

h) Tap water

i) Pond

j) Others

Specify .....

• **Household history of gastroenteritis**

13. Do you suffer from gastroenteritis?

a) Yes

b) No

14. If no, when did you experience gastroenteritis for the last time?

.....

15. Is there any household member who is suffering from gastroenteritis?

a) Yes

b) No

16. If no, when did any household member experience gastroenteritis for the last

time? .....

• **Use of animal waste**

17. Do you have a garden/ farm/ flowers?

- a) Yes
- b) No

18. If yes, what fertilizer do you use?

- a) Commercial
- b) Manure
- c) None
- d) Other fertilizers

Specify .....

• **Animal product consumption**

19. What animal products does the household consume?

- a) Beef
- b) Poultry
- c) Milk
- d) Eggs
- e) Others

Specify .....

• **Household area and activities**

20. What area of land does the household own?.....

21. What activities do household members engage in?

- a) Crop growing
- b) Trade

- c) Office work
- d) Livestock keeping
- e) Others

Specify .....

- **Contact with animal**

22. Do you have friends who keep cattle?

- a) Yes
- b) No

23. If yes, how often do you visit them?

- a) Every week
- b) Once a month
- c) Never
- d)

- **Crow access to household premises**

24. Do you see crows coming to the household premises?

- c) Yes
- d) No

That was the last question of our conversation. Thank you for your answers and time. I will visit you again for taking samples from the environment and people. Analysis of facts obtained from this household will be done together with the information from other households participating in this study. The outcomes of the study will be shared among stakeholders whenever available for the purpose of improving animal,

public and environmental health. Do you have any comment or question about the interview and our conversation?