2019-01

Assessment of risk factors and prevalence of campylobacter and salmonella in chickens under different production systems

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NM-AIST

https://doi.org/10.58694/20.500.12479/235

ASSESSMENT OF RISK FACTORS AND PREVALENCE OF *CAMPYLOBACTER* AND *SALMONELLA* IN CHICKENS UNDER DIFFERENT PRODUCTION SYSTEMS

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

January, 2019

ABSTRACT

The human population growth and increased urbanization in Tanzania, stimulates emerging of new livestock systems with variable intensification levels. In poultry production, traditional free-range backyard farming is now complemented by a range of intensive production systems. Intensification of poultry production may result in opportunities and threats with regards to food safety, *e.g.* in contamination of poultry with food borne pathogens such as *Campylobacter* species or non-typhoid *Salmonella* (NTS) species. The aim was to conduct cross sectional study across ten wards of Arusha district, northern Tanzania to assess risk factors and prevalence of these pathogens in emerging poultry production systems. Semi-quantitative analysis of chicken production systems with emphasis on biosecurity, health management practices and prevalence of food borne pathogens was done from September 2016 to January 2017. Interviews were conducted with 40 farmers, with equal representation of 4 production systems, 255 and 386 birds were screened for cloacae shedding of *Campylobacter* and NTS species respectively. Farm level prevalence of *Campylobacter* and NTS species was 57.7% (15/26) and 15% (6/40), respectively. Differences were observed between farms with regards to implementation of biosecurity and health management practices as well as use of extension services.

By contrast, prevalence of food borne pathogens was not farm-type specific, indicating that it is driven by other risk factors. Moreover, Multiple Component Analysis showed that risk factors associated with *Campylobacter* prevalence differ from those associated with *Salmonella*. Results can be used to inform on-farm food safety practices and the use of extension services, from all stake holders.

DECLARATION

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

21/01/2019

Emmanuel Sindiyo **Date**

Name and signature of candidate

The above declaration is confirmed by

22/01/2019

Dr. Gabriel Mkilema Shirima **Date**

Name and signature of supervisor

20/01/2019

Prof. Ruth Nicolet Zadoks **Date**

Name and signature of supervisor

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CERTIFICATION

The undersigned certifies that they have read and hereby recommend for acceptance by Nelson Mandela African Institution of Science and Technology a dissertation entitled: Assessment of risk factors and prevalence of *Campylobacter* and *Salmonella* in chickens under different production systems. The dissertation is submitted by Emmanuel Sindiyo in partial fulfillment of the requirements for the degree of Master of Life Sciences and bioengineering of the Nelson Mandela African Institution of Science and Technology Arusha, Tanzania.

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ACKNOWLEDGEMENT

I would like to express my sincere appreciation to those who contributed towards the successful completion of my dissertation work. Foremost, all praise is due to almighty God for His blessings and protection throughout this task. I primarily acknowledge my sponsors, The University of Glasgow and Nelson Mandela African Institution of Science and Technology (NM-AIST) through ZELS project for financial support and all logistics pertaining to my research activities. Special heartfelt gratitude to my supervisors: Professor Ruth N. Zadoks and Dr. Gabriel Mkilema Shirima for their fruitful contributions and positive criticism which polished the quality of my research output. There is no doubt that without their mentorship and guidance, I would have faced some challenges to effectively accomplish this work. Great thanks to Dr. Jackie Benschop from Massey University, for her support by providing her time for distance learning on R packages for data analysis. Also, special thanks to Dr. Kate Thomas and her team from KCRI laboratory, for their support during lab analysis. Their laboratory expertise and experience greatly made my laboratory analyses successful. Furthermore, exceptional thanks to Dr. Elingarami Nkya and Dr. Emmanuel Mpolya from the Department of Global Health and Biomedical Sciences at NM-AIST for their advice during planning process. Without them, my planning and data analysis process could have some challenges. Ultimately, I would like also to acknowledge the cooperation made by my fellow students; Ruth Maganga, Ancila Oiso Karani, and Aluna Chawala, without forgetting farmers, extension officers and Arusha City Director throughout my research work. Without their encouragement in some areas these activities would have been difficult for me. Also, I would like to acknowledge Mwailwa Smith Pangali, the director of Nsimbo district Council Katavi region for granting permission to pursue my studies.

DEDICATION

This dissertation is dedicated to my daughters; Sarafina Emmanuel and Invocavity Emmanuel for their love and daily prayers that made me strong throughout this work.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
BCB	-	Botoph Claydon, Buckingham
BPW	-	Buffered
CDC	-	Centers for Disease Control and prevention
DADPS	-	District Agriculture Development Plans
DED	-	District Executive Director
Dim.1	-	First dimension
Dim.2	-	Second dimension
D-SMS	-	District Subject Matter Specialist
FAO	-	Food and Agriculture Organization
GPS	-	Global Positioning System
h	-	Hours
HH	-	Household
HIV	-	Human Immunodeficiency virus
Indig	-	Indigenous
iNTS	-	Invasive Non-Typhoidal *Salmonella*
KCRI	-	Kilimanjaro Clinical Research Institute
LIA	-	Lysine Iron agar
MAFAP	-	Ministry of Agriculture Food and Agriculture Policies
MCA	-	Multiple Correspondence Analyses
mCCDA	-	modified Charcoal Cefoperazone Deoxycholate Agar
MSc	-	Master of Science
NIMR	-	National Institute for Medical Research
NTS	-	Non-typhoidal *Salmonella*
PPE	-	Personal Protective Equipment
QGIS	-	Quantum Geographic Information System
TSI	-	Triple sugar iron
UK	-	United Kingdom
URT	-	United Republic of Tanzania
USA	-	United States of America
WHO	-	World Health Organization
XLD	-	Xylose Lysine Deoxycholate

ZELS - Zoonoses and Emerging Livestock System

CHAPTER ONE

1.0 Introduction

1.1 Background

Tanzania has a population of 43.7 million chickens, 96% are local breeds (FAO, 2013). Chicken meat consumption is estimated to be 1 chicken per person per year and consumption is skewed to medium to high income populations in urban areas (Msami, 2007). Despite their important role in the economy and social life in Tanzania, poultry also exposes the person, environment and consumers to agents of zoonotic infections and food-borne illnesses. Non-typhoidal *Salmonella* (NTS) and *Campylobacter* spp. are two of the most important food-borne zoonotic pathogens, responsible for morbidity and mortality across the globe (Mañes-Lázaro *et al*., 2017). The global burden of food borne illness attributed to *Campylobacter* is estimated to be 96 million cases and 21 000 deaths annually while for NTS it is estimated to be 78 million cases and ~60 000 deaths annually (Havelaar *et al*., 2015). Studies conducted in Ghana on blood cultures of febrile children and other surveillances done in Sub-Saharan Africa identified *Salmonella* spp. as a major cause of blood stream infections *(*Al-Emran *et al.,* 2016). *Campylobacter* also plays a key role in causing diarrhoea in young children (Mason *et al.*, 2013). Such illnesses cost billions of US dollars in medical care and even result in death. Healthy poultry are considered as a potential source of both *Salmonella* and *Campylobacter* (FAO and WHO, 2009) infections in humans.

It has been documented that poultry at farm level are exposed to different risk factors such as contact with wild birds and rodents, poorly prepared feed, contaminated water, flies, poor farm structures and other husbandry practices (Arsenault *et al*., 2007). Risk factors at the farm level that are associated with the occurrence of *Salmonella* and *Campylobacter* in chickens from different farming system in Tanzania are not well documented. The main objective of this study was to assess risk factors at farm level that are associated with the occurrence of *Campylobacter* and *Salmonella* species in chickens reared in different production systems in Arusha District, Northern Tanzania.

1.2 Problem statement and Rationale

From animal farms to the commercial production of food commodities there are numerous possibilities for transmission of *Campylobacter* and *Salmonella* spp. infections through cross-

contamination. Infection with *Campylobacter* spp. from consumption of poultry is the leading cause of human food borne illness in the world (Epps *et al.*, 2013). In Morogoro Tanzania, *Campylobacter* spp. was found prevalent in children less than five years (Chuma *et al.*, 2016), while NTS infection is an important cause of febrile disease among hospitalized children in rural Tanzania (Mtove *et al.*, 2010). Many of the current risk assessment models start at the point of estimating the prevalence of contaminated *Campylobacter* and *Salmonella* positive birds as the birds enter the slaughterhouses. This means that on-farm control strategies are not well investigated (WHO, 2009). Risk factors associated with occurrence of *Campylobacter* and *Salmonella* species in chickens in different production system are not well investigated and documented. Only fragmented reports with limited sample sizes and farm type are available. Therefore it was important to obtain this information from existing four production systems in order to understand how risks contribute to occurrence of food borne pathogens, *Salmonella* and *Campylobacter* at farm level.

1.3 Objectives

1.3.1 Overall objective

Determine farm level associated risk factors and prevalence of *Campylobacter* and *Salmonella* spp. for better understanding of its distribution and eventual control strategies in four Tanzanian chicken farming systems.

1.3.2 Specific objectives

(i) To assess prevalence of Salmonella and Campylobacter spp. in the four chickens production systems

(ii) To assess farm characteristics based on biosecurity, health management and extension service delivery in the four chickens production systems

(iii) To determine the association between occurrence of Campylobacter, Salmonella spp. and risk factors in the four chickens production systems

1.4 Research questions

(i) What is farm level prevalence of *Campylobacter* and *Salmonella* species in four chicken production systems?

(ii) What are farm characteristics based on biosecurity, health management and extension service delivery in the four chicken production systems?

(iii) What are farm characteristics based on biosecurity, health management, extension service provision that are associated with prevalence of *Campylobacter* and *Salmonella* in chicken?

1.5 Significance of the study

The information from this study is needed to propose measures that could improve poultry management practices and reduce risk of food borne pathogens to people along the food chain.

CHAPTER TWO

2.0 Literature review

2.1 Poultry production in Tanzania

Poultry farming is one of the major economic activities in Tanzania with 43.7 million chickens and an annual growth rate of 5.1%. About 96% are the local breeds (MAFAP, 2013). These chickens are raised in existing different production systems which are traditional and commercial production system (Goromela, 2008). The commercial system of chicken production is based on improved ecotypes (layers and broilers) and is found in the urban and the peri urban settings. The traditional small holder poultry production is characterized by small to medium numbers of local chicken ecotypes for the purpose of income generation, food security and employment to women and children and also supplies most of the meat and eggs consumed in the rural and 20% in urban areas (Msami, 2007).

Demand for poultry meat and eggs for protein supplementation are growing very fast due to population growth and increased purchasing power (Nonga *et al.*, 2010). These changes lead to alterations in the poultry production systems, which also lead to utilizing larger scale and more intensive production, centralized processing, and wide-scale distribution. Poultry products must be microbiologically safe for consumption to avert human food borne illness, however both commercial and traditional poultry systems are sources of *Campylobacter* and *Salmonella* pathogens (Kiilholma, 1999).

2.2 Overview of *Campylobacter*

2.2.1 Infectious agent

The genus *Campylobacter* is a diverse group of 15 species and 6 subspecies (Taylor, 2012). They are gram-negative, non-spore forming bacteria (Dylan *et al.*, 2014). The most important species of *Campylobacter* are the thermophilic species: *C. jejuni* subsp. *jejuni*, *C. coli* and *C. lari* (Hörman and Hanninen, 2012). These species are classified "thermophilic" since they grow at 42°C but not at 25°C (Penner, 1988). *Campylobacter* generally appear curved or comma-shaped, and are able to move via unipolar or bipolar flagella (Veron and Chatelain, 1973). They are microaerophilic, can be cultured at the environment of 5% oxygen, 10% carbon dioxide, 5% hydrogen gas and 80% Nitrogen gas (Aines *et al.*, 2011). When exposed to atmospheric oxygen, *C. jejuni* is able to change into a coccal form (Jones *et al.*, 1993).

Campylobacter jejuni are positive to both oxidase and catalase tests (Aines *et al.*, 2011) and are non fermentative bacteria (Barrett *et al.*, 1988). The usual habitat of *Campylobacter* species is the mucosal layer that coats the crypts of the intestinal epithelia of mammals and birds (Taylor, 2012). They are best cultured at 42 °C, its survival at room temperature is poor, but *Campylobacters* can survive for a short time at refrigeration temperatures up to 15 times longer at 2°C than at 20°C. They grow quite slowly (72-96h) and are resistant to cephalothin (Allos, 2001).

2.2.2 Epidemiology

The global incidence of campylobacteriosis has increased in the past decade and the number of cases has increased in North America, Europe and Australia (Kaakoush *et al.*, 2015). Humans contract *Campylobacter* from contaminated food or water and poultry are considered as major reservoir of *Campylobacter* spp. (Sahin *et al.*, 2001). Possible sources of *Campylobacter* infection for chickens include feed, water, staff, wild birds, rodents, insects and air (Pattison, 2001; Arsenault *et al.*, 2007).

The infectious dose to cause illness in humans is as low as 500 cells (CDC, 2015). Most of Developing countries do not have national surveillance programs for food borne illnesses such as campylobacteriosis; therefore, incidence values in terms of number of cases for a population do not exist (Coker *et al.*, 2002). *Campylobacter jejuni* infections are hyper endemic among young children and those who are elderly in tropical developing countries (Kishan and Nyati, 2013). Thermophilic *Campylobacter* were isolated from 11.4% of the screened individuals (n=1195) in Eastern Tanzania (Komba *et al.*, 2013). The prevalence of *Campylobacter* in raw milk and beef carcasses in Tanzania was 9.5% and 13.4% respectively (Kashoma *et al.*, 2016) and 32.5% in pig faeces (Kashoma *et al.*, 2015). In Morogoro Tanzania, the prevalence of thermophilic *Campylobacter* in children was 19% while in broiler chickens it was 50% (Chuma *et al.*, 2016).

2.2.3 Clinical presentation

Patients with *C. jejuni* or *C. coli* experience acute watery or bloody diarrhoea, fever, weight loss and cramps that last an average of 6 days and onset of symptoms usually occurs 24 to 72 hours following ingestion and may take longer time to develop in those infected with a low dose. Men and women are equally affected (Zumla, 2010).

Campylobacter jejuni is the commonest species found in the poultry but is not currently considered to be pathogenic to chickens (Blaser, 1997).

2.2.4 Diagnosis

Diagnosis is by direct examination of a stool sample using contrast microscopy or gram stain (Zumla, 2010) and confirmation is done by stool culture. PCR protocols, for example multiplex PCR, are used for the direct detection and differentiation of *C. jejuni* and *C. coli* in stools (Al Amri *et al.*, 2007).

2.2.5 Prevention and control

Control of campylobacteriosis occurrence in human relies on general hygienic measure at all level of food chain from primary production to retail (Giangaspero, 2013), including good hygienic practices at household level (Lin, 2009). In poultry, measures to reduce *Campylobacter* include enhancing biosecurity to avoid transmission from the environment to the flock at farm level (Newell *et al*., 2011). It is widely accepted that contamination of poultry by *Campylobacter* is a significant risk factor of human campylobacteriosis and therefore, prevention and control of *C. jejuni* in poultry would reduce the risk of human exposure to *Campylobacter* and is an important food safety issue. Biosecurity measures are practical (Lin, 2009), hygiene procedures should be followed to promote better health and well being of human from this pathogens.

2.3 Overview of *Salmonella* infection

2.3.1 Infectious agent

Salmonella spp. are motile gram-negative facultative anaerobic bacteria in the family of *Enterobacteriaceae*. The *Salmonella* genus consists of two species, *Salmonella enterica* and *Salmonella bongori*. Most pathogenic species of *Salmonella* causing illness in human belong to the *Salmonella enterica* species (Makendi *et al.*, 2016). This species is further divided into 6 subspecies: *Salmonella enterica* subspecies *enterica, salamae, arizonae, diarizonae, houtenae* and *indica* (Kong, 2011).

They are over 2500 serotypes (John *et al.*, 2012) and Salmonella enterica serotype Enteritidis and Typhimurium are the most causing agent of non typhoidal salmonelosis in human.

2.3.2 Epidemiology

The primary route of *Salmonella* infection in human and animals is through fecal oral transmission (Ruby *et al.,* 2012). The bacteria have to pass through the alimentary system and survive the acidic environment of the stomach (Foley *et al.*, 2013). In sub-Saharan Africa, invasive NTS (iNTS) is endemic and considered as a leading cause of bloodstream infections (Marks *et al.,* 2017)**.** NTS causes sepsis and deaths in immune suppressed patients (Waldner *et al.,* 2012). In Zaire, four percent of the total pediatric admissions had bacteraemia due to NTS (Green and Cheesbrough, 1993). In Malawi, 37% (449) of adults admitted to hospital were NTS blood culture positive (Gordon *et al.,* 2001), while in Tanzania iNTS was common in areas where malaria transmission was intense (Biggs *et al.,* 2014). Outbreaks caused by several kinds of *Salmonella* spp were reported in the USA and epidemiologic trace back and laboratory findings link the outbreaks to contact with poultry (CDC, 2017). Poultry products are one of the most important sources of human infection (Tauxe, 2002). Contamination of poultry carcasses with *Salmonella* seems to be mostly linked to flock contamination during rearing and/or transportation to slaughter. Risk factors for flock colonization by *Salmonella* include country of origin, origin of the feed and number of birds per flock (Franz *et al.,* 2008), and presence of rodents in the farm (Meerburg and Kijlstra, 2007).

2.3.3 Clinical presentations

Gastroenteritis is the most common clinical presentation of NTS infection (Chen *et al.*, 2013). Clinical manifestations of nontyphoidal salmonellosis include diarrhoea, bacteraemia, nausea, vomiting, endovascular infections, and localized infections. Following ingestion of contaminated food, clinical symptoms of diarrhoea appear after 6-48 hours post exposure (Crum-Cianflone, 2008). Sometime the illness may be self-limiting within 4-7 days (Foodborne Illnesses Fact Sheets, 2004). Serotypes such as Dublin and Choleraesuis may be very invasive resulting in severe infections and deaths in infants, older persons and people with immunosuppressive conditions including HIV, haemoglobinopathies, and malignant neoplasms (Feasey *et al.,* 2012)

2.3.4 Diagnosis

Culturing organisms continues to be the mainstay of clinical diagnostic testing for NTS infection (Crump *et al.*, 2015). Although culture-independent diagnostic tests are increasingly used by clinical laboratories to diagnose *Salmonella* infection, isolates are needed for serotyping and antimicrobial susceptibility testing. Other tests include Deoxyribose Nucleic Acid (DNA) detection using polymerase chain reactions (PCR) (Tennant *et al.*, 2011).

2.3.5 Prevention and control

No vaccine is available against NTS infection in humans (Ferreira, 2015). Preventive measures are aimed at avoiding foods and drinks at high risk for contamination; frequent hand washing, especially after contacting animals or their environment (WHO, 2006). Prevention and control strategy in poultry include adopting Good Agricultural Practices (GAP) and Hazard Analysis Critical Control Point (HACCP) but also hygiene and biosecurity procedures in poultry production, flock culling, and product diversion to processing (World Organisation for Animal Health [OIE], 2010). In the UK, the occurrence of salmonellosis was reduced significantly when vaccination of chickens was introduced. The vaccination of chickens in Europe targets *Salmonella enteritidis* and *Salmonella typhimurium*, because those two serovars were most common in people often came from poultry (The British Egg Industry Council, 2013).

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CHAPTER THREE

3.0 Materials and Methods

3.1 Description of Study Area

Arusha district is one among seven (7) districts of Arusha region. It lies between Longitudes 34.5 º – 38˚E and Latitudes 2˚- 6 ˚S, with 25 wards. According to national census data of 2012, Arusha district has human population of 41 6442 (19 9524 male, 21 6918 female) with an average of 4 occupants per household. It has 2 2898 dairy cattle, 3 1173 indigenous cattle, 3 1378 goats, 1 5567 sheep, 2 9651 pigs, 1200 rabbits, 1 2300 broilers, 9500 layers, 5200 indigenous chickens and 2 651 other birds (DADP report, 2013/14). Existing farming systems for poultry production in Arusha District are intensive broiler, intensive indigenous, and semi intensive indigenous and extensive indigenous (free range).

3.2 Study design and sampling

A cross-sectional survey was conducted by questionnaire administration at household level followed by collecting chicken samples (cloacal swabs).

3.2.1 Selection of district and wards

Arusha district was selected based on the fact that it is an area where the University of Glasgow in collaboration with Nelson Mandela African Institution of science and Technology (NM-AIST) Arusha is implementing its Zoonosis and Emerging Livestock Systems (ZELS) programme, the presence of four poultry production systems and proximity to the Kilimanjaro Clinical Research Institute (KCRI) where laboratory work was conducted. An introductory letter was written by the university NM-AIST to the Arusha district Executive Director (DED) requesting permission to conduct research in the area of jurisdiction.

Selection of wards was done at district level with the help of poultry subject matter specialists (PSMS). Out of 25 wards, 20 were selected by excluding 5 wards which do not have the four farming systems. Then 10 out of the remaining 20 wards were randomly selected by writing names of the Wards in piece of papers and picked from the box with replacement. The selected wards were: Kimandolu, Moshono, Themi, Lemara, Engutoto, Sinon, Terat, Muriet, Sombetini and Elerai.

3.2.2 Selection of household farmers

For every selected ward, field extension officers were asked to produce a list of poultry farmers stratified based on the type of poultry farming systems (Intensive Broiler, Intensive indigenous, Semi intensive indigenous and Extensive indigenous). Then in collaboration with ward livestock extension officers list of farmers per production system was provided and one farm selected at random from each system (Fig.1).

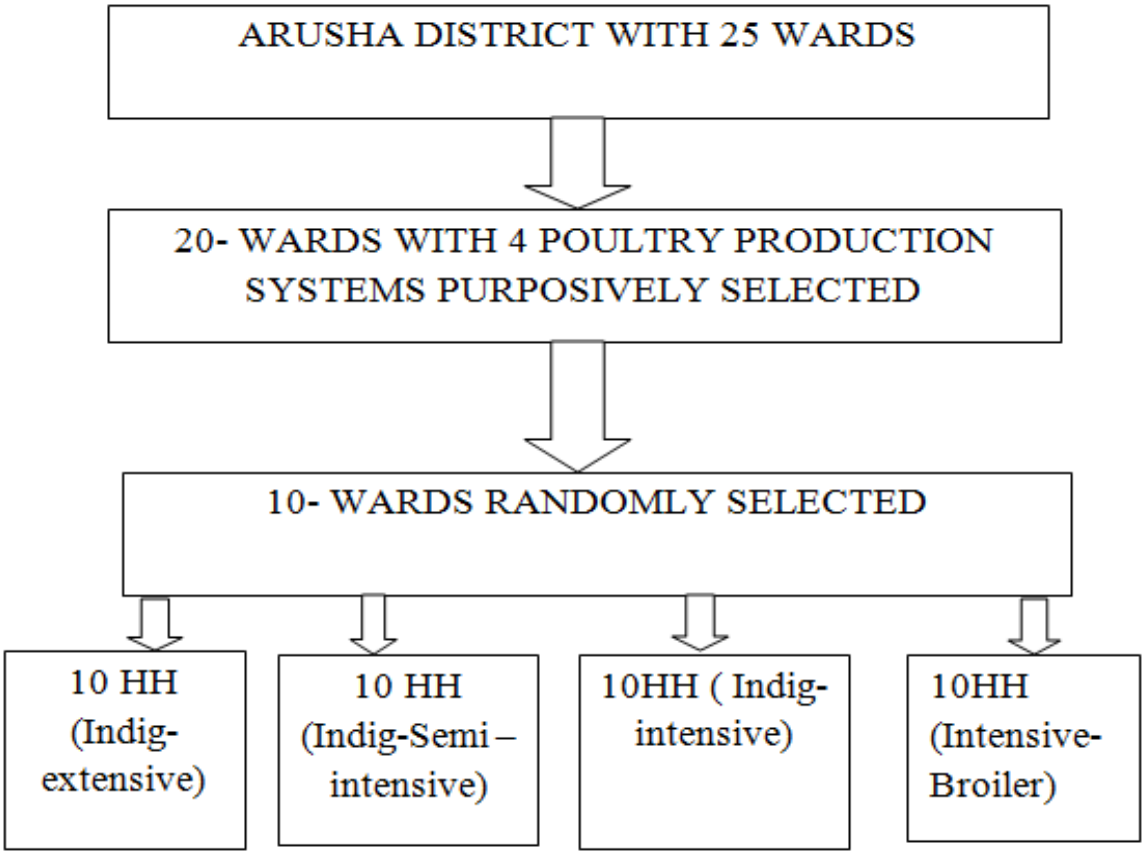

Figure 1: Sampling design for selection of poultry farms in Arusha district

HH= house hold

Indig.=Indigenous

3.2.3 Selection of chickens

Purposive sampling was used to select birds from the flock. All chickens were selected for the farm which had less than 10 chickens.

3.3 Data collection

3.3.1 Questionnaire and geographical information points (GPS)

Since this study was part of a ZELS project the questionnaire related to husbandry practices was developed and discussed by different experts within the project to capture the major risk factors. The questionnaire was semi structured (Appendix 1) and designed to address management related risk factors including sources of household income and farming systems. Pilot testing was done using four household farmers at Sokon - 1 ward, one in each production system. By testing the questionnaire we managed to know about the clarity of questions, potential difficulties farmers had in answering them, and the time required to fill it out, then questions were updated. Farmers were contacted by mobile phone so that they could slot a time for the interview session. The questionnaire was administered to 40 farmers after getting their verbal consent. Questionnaire administration was conducted 3 days prior to sampling of chickens.

The geographical point for each household was collected by using a GPS device (GARMIN-e Trex 10) and recorded in an Excel spreadsheet (Microsoft, Seattle, USA) for future analysis.

3.3.2 Sample collection

(i) Chicken handling

Chickens for cloacal swabbing were handled gently to avoid any injury, in accordance with the Animal Welfare Act no19 of 2008, part V section 40 to 48. Also ethical clearance was obtained from KCRI (certificate No. 832) and the National Institute of Medical Research (NIMR) (Ref no NIMR/HQ/R.8C/VOL II/653). Environmental sample were collected by using boot swabs.

(ii) Cloacal swabbing and environmental sample collection

Cloacal swabs were collected by inserting the entire tip of the swab into the cloaca, while the tip of the swab was inside the cloacae gentle pressure against the mucosal surface was applied along with circular motions during swabbing. Each chicken was swabbed twice, once with a charcoal Amies swab (black agar storage swab) and one with a plain Amies swab (clear agar swabs). Both swabs were supplied by Thermo Fisher Scientific, Newport, UK. The swab was removed gently and immediately closed in the respective Amies tubes, labeled and preserved into cool boxes before transported to the laboratory for analysis.

Environmental samples were collected by using one pair of boot socks (Sterile Boot Cover Swab for sampling poultry housing (BTSW Series), Solar Biologicals Inc., Ogdensburg, USA), by walking in the four directions inside the chicken house. The boot socks were removed and stored in stomacher bags. After collecting environmental samples and cloacal swabs before visiting the next farm all disposable personal protective equipment (PPE) was changed and boots were disinfected using 70% ethanol. All samples were transported in a cool box to the laboratory within 0500 hours (from 1st sampled chicken to the laboratory).

3.4 Isolation, identification and confirmation of *Campylobacter* and *Salmonella* spp

3.4.1 *Campylobacter* spp.

Campylobacter culture was initiated on the day of sample collection. Amies charcoal swabs were removed from their transport containers and tips removed aseptically by cutting them off into a plastic universal tube containing 20 mL Bolton broth (Oxoid, Basingstoke, UK) supplemented with 5% laked horse blood (TCS Biosciences, Botoph Claydon, Buckingham, UK) and selective supplement SR0208E (Oxoid), vortexed aseptically for 10 sec and placed into a micro-aerophilic jar with CampyGen sachets (Oxoid). Samples were incubated at 37 ± 2°C for at least 0400 h before being moved to 42 ± 2 °C for a further 42- 46hrs, and then plated onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid) plates and incubated at 42 ± 2 °C in a micro-aerophilic jar with appropriate volume CampyGen sachet for 4800 h. Plates were examined for typical *Campylobacter* colonies, i.e. moist, flat, shiny, round and grey to creamy grey colonies. Suspect colonies were subcultured onto Columbia blood agar (Oxoid), incubated microaerophilically at 42 ± 2 °C for 48 h and were subjected to oxidase and catalase testing and Gram stain for confirmation. Detection and confirmation of *Campylobacter was* done by KCRI staff.

3.4.2 *Salmonella* spp.

Samples for *Salmonella* detection were stored overnight in a refrigerator between 2 and 8°C. Tips were aseptically removed from the plain Amies swabs the next day, placed in 20 mL buffered peptone water (BPW) (Oxoid), vortexed for 10 sec and incubated at 37 ± 2 °C for 18- 20 hrs. A small volume (0.1 mL) of the enriched BPW was then transferred into 10 mL of Rappaport-Vassiliadis Soya Peptone (RVS) (Oxoid) broth and incubated at 42 ± 2 °C for 24 h. One loopful (10 µL) of enriched RVS was transferred onto xylose lysine deoxycholate agar

(Oxoid) with 5 µg/mL novobiocin (Sigma-Aldrich, St. Louis, MO, USA) (XLD+N) and streaked for isolation. *Salmonella* were examined on XLD+N plates for typical colonies, which appear as red colonies with or without a black centre. At least 2 typical colonies per XLD+N plate were streaked onto MacConkey agar (Oxoid) and incubated overnight at 37 ± 2 °C. Lactose fermenting colonies, i.e. those with pink appearance, were discarded and non-lactose fermenting colonies were individually transferred into 5 mL tryptone broth (Oxoid) and incubated at 37 ± 2 °C for 4 to 24 h. Growth from the broth was inoculated onto MacConkey agar to check for purity and stabbed into lysine iron agar (LIA) (Oxoid) slopes and triple sugar iron (TSI) (Oxoid) slopes to assess phenotype.

All media were incubated overnight at 37 ± 2 °C. Kovacks' indole reagent (Merck KGaA, Darmstadt, Germany) was added to the incubated tryptone broth to test for indole production. Presumptive identification of *Salmonella* spp. isolates was based on negative results in the indole test, alkaline slant and butt (purple colour) in LIA, and red slope with yellow butt and gas production in TSI. Identity was confirmed by testing with poly-H and poly-O agglutination tests (Statens Serum Intitut, Copenhagen, Denmark) and Microbact 12A (Oxoid) test strips following the manufacturers' instructions. Detection and confirmation of *Salmonella* was conducted by the KCRI laboratory.

3.5 Data analysis

Data were stored and checked for missing values and outliers in Excel (MicroSoft, Seatle, WA), with additional processing using Excel (visual analysis), Statistix 10 (Analytical Software, La Jolla, CA) or R Studio software (R Studio, Boston, MA; quantitative analysis). To test for an association between farm type and categorical variables (e.g. biosecurity characteristics, health management, socio-economic parameters) Chi-square analysis was used whilst one-way ANOVA was applied for continuous variables (e.g. mean number of chickens or chicken houses per farm). Statistical significance was declared at $p < 0.1$ to avoid potential loss of power associated with the limited number of farms per farm type. To uncover relationships between categorical variables and visualize clustering of variables, multiple correspondence analysis (MCA) (Sourial *et al.*, 2010) was conducted using the Facto MineR, Facto Extra and ggplot2 libraries in R. Production system was excluded from the MCA and visualized in the plots after the analysis had been run. QGIS software version 2.18.3 was used to generate a map of the study area showing the production system and culture results for each farm. Ranking of farm types was done based on biosecurity and health

variables only, in which a farm type was assigned a number for rank based on whether the farm type showed the best behavior for a particular characteristic (rank 1), the 2nd best behavior (rank 2), the 3rd best behavior (rank 3) or the worst behavior (rank 4). If two or three farms had the same rank the rank were averaged. If second and third were the same both were given 2.5 (average of rank 2 and 3rd), but if one was better than the rest and the rest were all the same rank 3 were provided (Average Rank 2,3 and 4=3).

For example, mixing of chickens with other host species never occurred in broiler farms (best biosecurity, rank 1), it occurred at equal frequency in intensive and semi intensive indigenous farms (both ranked at 2.5) and it was most common in extensive farms (worst biosecurity, rank 4). Ranks were assigned for several biosecurity characteristics (Mixing with different age, Mixing with other species, contact with wild bird, contact with rodents, physical barrier, dedicated boots, foot dip, rodent control, deep litter) so that the average biosecurity ranking could be compared across farm systems. The same approach was used for general health management characteristics. Kruskal-Wallis one way Non parametric ANOVA for Ranking were employed to rank the farms in R studio.

3.6 Feedback session

After data analysis we communicated important findings to farmers and field extension officers. Participatory approach such as group discussion and presentations were used to convey the information.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Results

A total of 40 farms were visited (10 farms per production system), 386 chickens out of the target number of 400 (100 birds per production system) were sampled and cloacal swabs collected. These were intensive broiler (n=99), Intensive indigenous (n=99), Semi intensive (n=98) and extensive (n=90). Due to mortalities which occurred prior to actual sampling dates, fewer chickens than planned were sampled in indigenous chicken farms. Less sampling in broiler farms was due to the fact that chicken were sold prior to actual sampling date. Collection of environmental swabs was successful in all 40 farms.

4.1.1 Farm characteristics based on continuous variables

The mean number of chickens available per house hold farm was 715 for broiler, 199 intensive indigenous, 57 for semi intensive and 39 in extensive farms respectively. The number of birds by production system increases as level of intensification increases (Fig. 2).

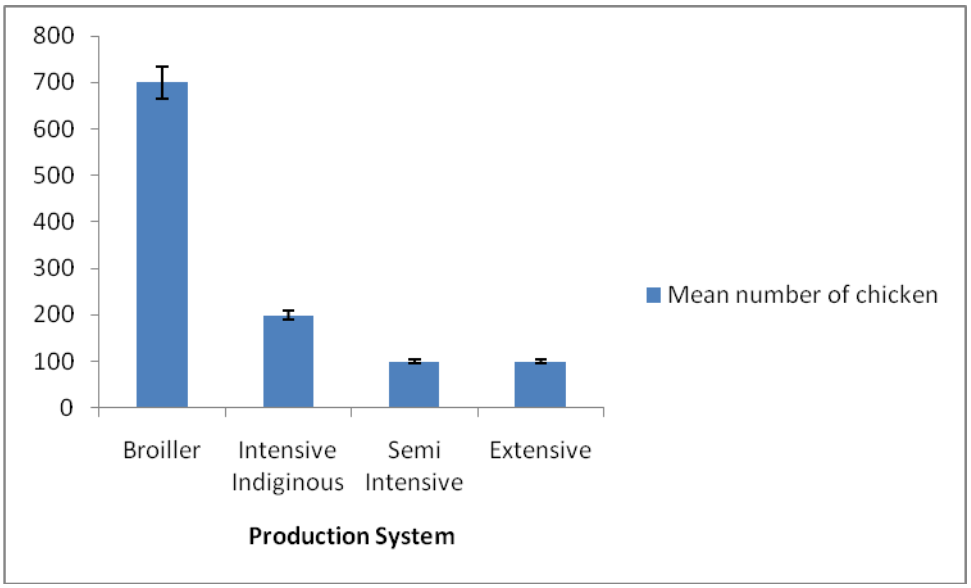

Figure 2: Mean number of chickens per household by production system (bars indicate standard error)

4.1.2 Occurrence of *Salmonella* and *Campylobacter* spp.

(i) Salmonella

Out of 40 farms in which chickens were sampled 5 were positive to *Salmonella*. The number of *Salmonella* positive farms was numerically but not statistically higher on intensive farms (broilers or indigenous chickens) than on non-intensive farms (Fig. 3).

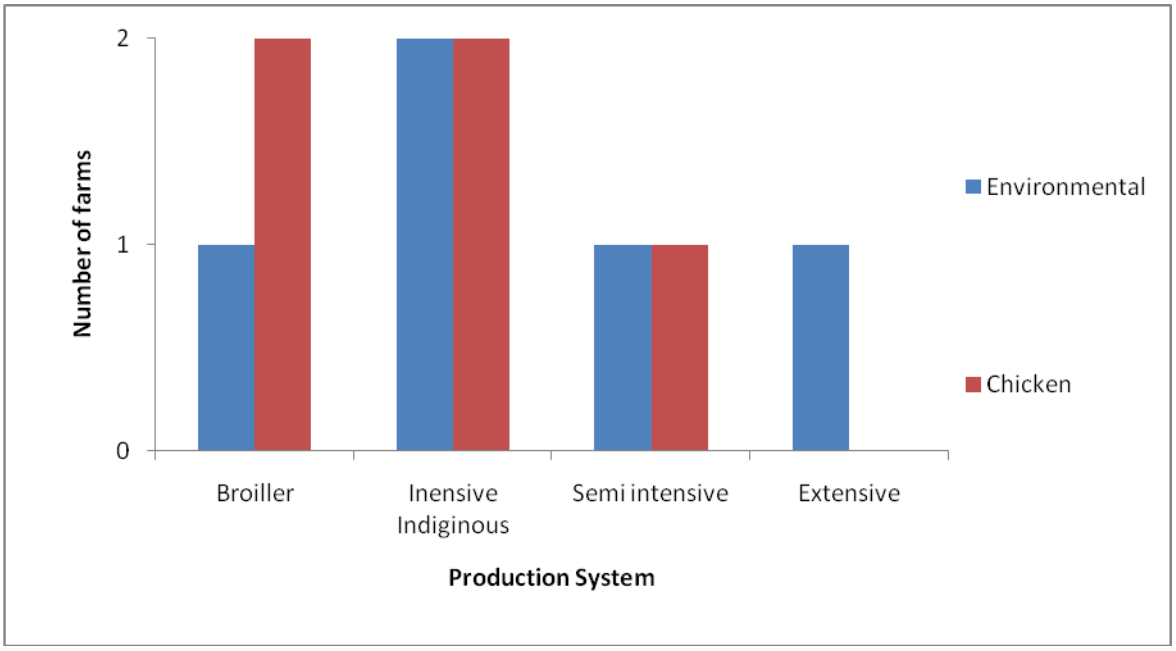

Figure 3: Chicken and environmental *Salmonella* farm status per production system
Note; Among 5 *Salmonella* positive farms, four had environmental samples that were also positive to *Salmonella*. One extensive farm did not have positive results for cloacal swabs but was *Salmonella* positive in the environment.

(ii) Campylobacter

Out of 26 tested farms, 15 were positive to *Campylobacter*. The number of *Campylobacter* positive farms was numerically higher on farms with indigenous chickens (intensive, semi-intensive or extensive) than on broiler farms. The number increases as intensification level increases in indigenous (Fig. 4). The distribution of *Salmonella* positive farms and *Campylobacter* positive farms across wards and production systems is shown in Fig. 5.

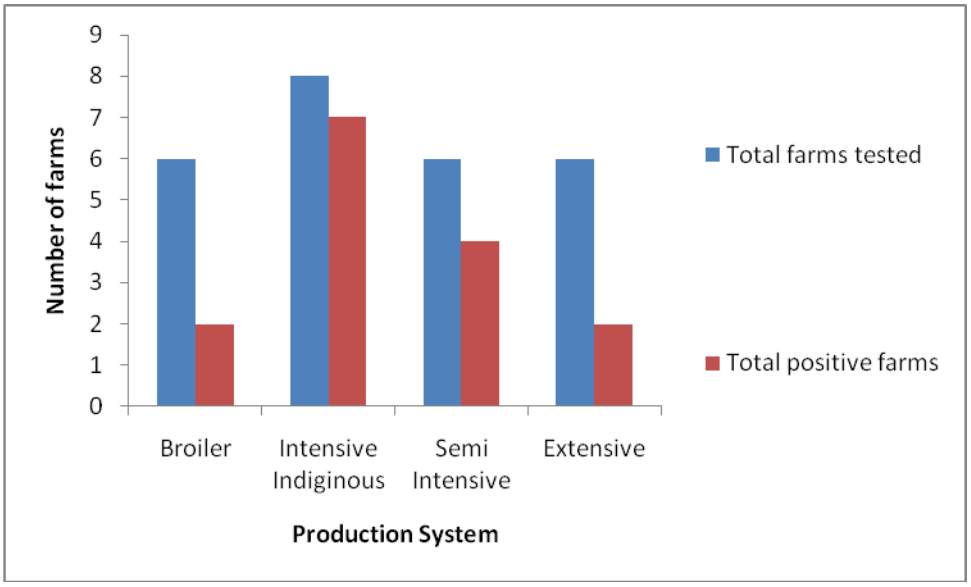

Figure 4: Distribution of *Campylobacter* positive farms among the four poultry production systems

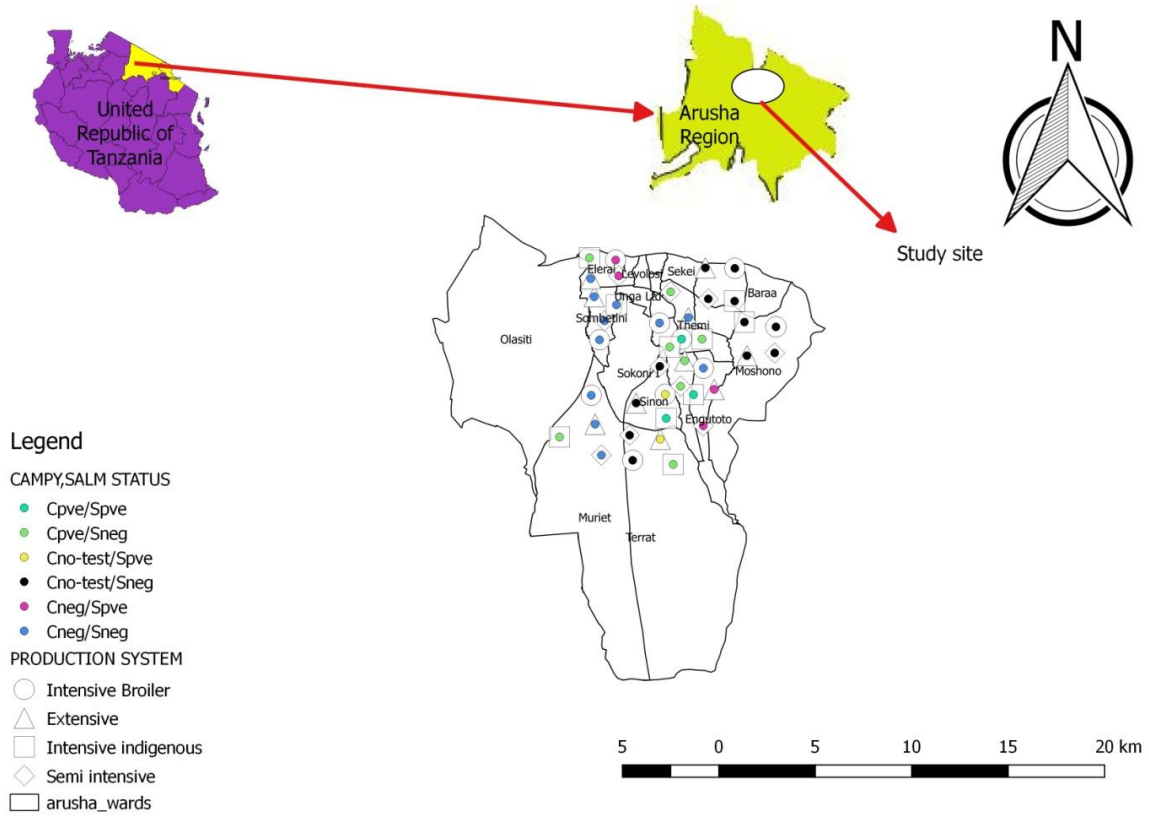

Figure 5: Map of Arusha District showing production system and disease status
Note; Cpve-*Campylobacter* positive farms: Spve-*Salmonella* positive farms: Cno-farms in which *Campylobacter* was not tested: Cneg-farms negative to *Campylobacter* test: Sneg-*Salmonella* negative farms)

4.1.3 Farmers social and economic activities

(i) Farmers' main source of income

The minority of farmers (17 of 40) depended on poultry production as their main source of income (Table 1). The proportion of farmers that depended on poultry production for income was numerically higher among broiler farmers than among other farmers, the difference between farm types was not statistically significant ($p>0.1$).

(ii) Farmers' skills in poultry production

The mminority of farmers (13 of 40) said to have skills in poultry production. The number of farmers with skills was lower in broiler farms and extensive farms, but the numerical difference was not statistically significant (Table 1).

(iii) Gender issues in Chicken Management

Despite of the fact that 20% of the farms are owned by women and 80% by men, only 30 % are managed by men while 30% of the income from sale of chickens and its product were directly used by women (Table 1).

Table 1: Demographic and social economic information for poultry farms in Arusha District, Tanzania (n=10 per production system type)

Variable description (Yes/No)	Production system				Pearson Chi-square p-value	Pearson χ^2 value
	Intensive broiler	Intensive indigenous	Semi – intensive indigenous	Extensive indigenous		
Age 20-29	0/10	0/10	1/9	1/9	0.55	2.105
Age 30-39	2/8	1/9	3/7	1/9	0.59	1.897
Age >40	8/2	9/1	6/4	8/2	0.44	2.728
Gender(F/M)	2/8	3/7	2/8	1/9	0.74	1.250
Farm management						
F/M	8/2	7/3	7/3	6/4	0.81	0.9524
Provide money for inputs F/M	4/6	4/6	5/5	2/8	0.57	2.0267
Main source of income						
Chicken production	3/7	0/10	1/9	1/9	0.44	2.707
Crop production	1/9	0/10	1/9	1/9	0.38	3.0770
Business	2/8	3/7	3/7	4/6	0.50	2.3820
Civil servant	1/9	3/7	1/9	0/10	0.53	2.2220
Both(chicken& crop)	2/8	4/6	3/7	3/7	0.81	0.9524
Other	1/9	0/9	1/9	1/9	0.81	0.97500
Money after sale chicken and its products F/M	37	3/7	2/8	4/6	0.81	0.95238
Farmers' level of education						
Standard seven	2/8	3/7	3/7	5/5	0.54	2.1652
Form four	4/6	3/7	2/8	2/8	0.71	1.3793
Form six	3/7	1/9	3/7	1/9	0.48	2.5000
Diploma	1/9	0/10	0/10	0/10	0.38	3.0770
University	0/10	3/7	1/9	1/9	0.23	4.3470
Adult education	0/10	0/10	1/9	1/9	0.55	2.1053
Farmers with skills in poultry production	1/9	2/8	5/5	5/5	0.12	5.8120

4.1.4 Biosecurity characteristics of poultry farms in Arusha District

On the majority of farms (24/40), chickens of different ages were mixed. However, this was least common on broiler farms and increasingly common as the farming system became less intensive. Similarly, mixing of chickens with other animal species and contact with wild birds was least common on broiler farms and increasingly common as the farming system became less intensive (Fig. 6).

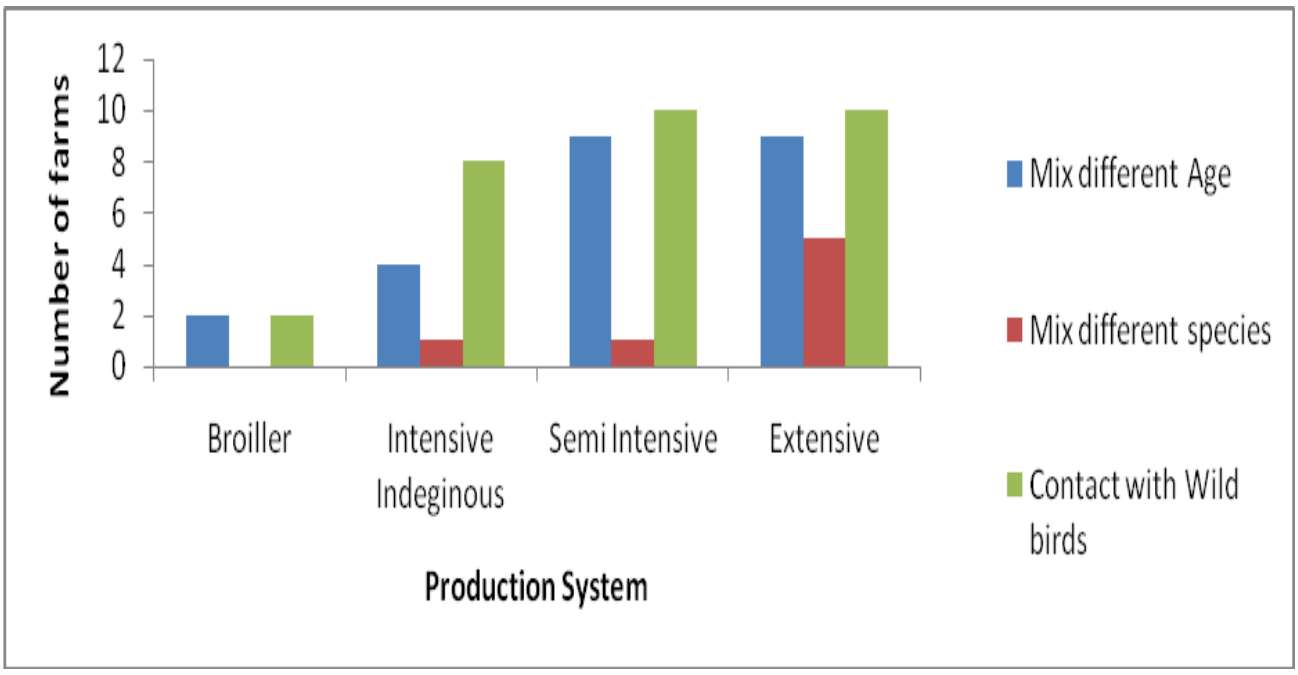

Figure 6: Number of farms that mix chicken (multi-age, other species) or contacted with wild birds by production system

Out of 40 farmers interviewed 16 sold clinically sick chickens to others. The business was commonly observed in indigenous chicken (extensive 7/10, semi intensive 6/10, intensive 3/10), with no such phenomenon in broiler farms. Destroying of dead chickens was observed in 18 of 40 farms. The number of farmers who destroy dead chickens is higher in broiler farms, level of practice decreases with intensification (Table 2).

(i) Physical aspect of biosecurity

Use of dedicated boots by farm attendants differs numerically between farm types; it is most common on broiler farms (Table 2). However use of foot dip was observed in only one broiler farm. A significant different in the use of deep litter systems was observed across the four production system which display the following order (Broiler>Intensive indigenous>Semi intensive indigenous>Extensive indigenous). Rodent control did not differ significantly between farms (Table 2).

Table 2: Biosecurity characteristics of poultry farms in Arusha District, Tanzania (n=10 per production system type)

Variable description (Yes/No)	Production system				Pearson Chi-square *p*-value	Pearson χ^2 value
	Intensive broiler	Intensive indigenous	Semi – intensive indigenous	Extensive indigenous		
Mixing or contact with						
different age	2/8	4/6	9/1	9/1	0.001	15.833
other species	0/10	1/9	1/9	5/5	0.017	10.216
wild birds	2/8	8/2	10/0	10/0	0.0000	22.933
Rodents	9/1	10/0	10/0	10/0	0.38	3.0769
Presence of security barriers						
physical	9/1	9/1	8/2	5/5	0.104	6.1649
dedicated boots	4/6	1/9	0/10	0/10	0.020	9.8286
barrier foot dip	1/9	0/10	0/10	0/10	0.38	3.0769
Rodents	2/8	2/8	0/10	0/10	0.25	4.0892
Use of deep litter system	10/0	4/6	3/7	1/9	0.0004	18.182
Handling of sick chickens						
Sale chicken with clinical signs	0/10	3/7	6/4	7/3	0.0058	12.5000
Slaughter	1/9	1/9	2/8	3/7	0.58	1.9558
Destroy un recovered chicken after treatments	9/1	6/4	2/8	1/9	0.0009	16.5600
Supervision of farm activities						
Employ Labor at any time	5/5	1/9	2/8	0/10	0.03	8.7500
Men/Woman	4/6	3/7	7/3	9/1	0.03	9.3095
Whole family	0/10	0/10	0/10	5/5	0.0007	17.1430
Cleaning, feeding and drinking water provision						
Father	2/8	3/7	3/7	2/8	0.84	0.8400
Mother	8/2	7/3	7/3	3/2	0.098	6.2933
Whole family	0/10	0/10	0/10	5/5	0.0007	17.1430

(ii) Farm ranking based on biosecurity characteristics

Among the four farm types there was statistical significant difference (p<0.1) in the mean rank of farms based on biosecurity measures (presence of security barriers for rodent control, use of foot dip, use of deep liter system, mixing of chicken of different group/mix chicken of different species in one pen etc), with broiler farms ranked first and extensive farm the worst in biosecurity (Fig. 7; Appendix 4).

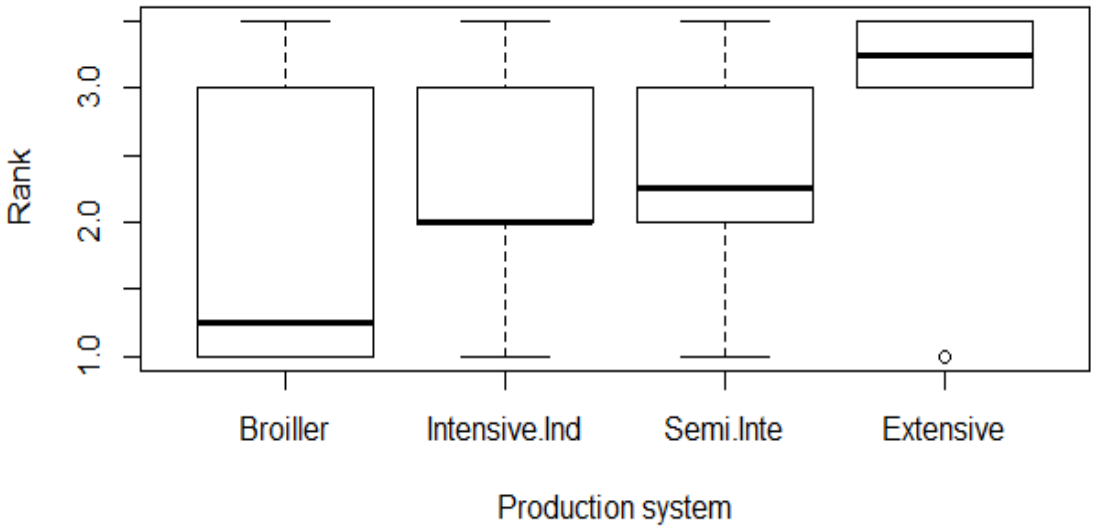

Figure 7: Box plot of Farm Rank based on biosecurity in the four production systems.
Note; Broiler= "1st Best", Intensive indigenous = "2nd Best", Semi intensive = "Good'', Extensive= "Worst").

4.1.5 Health management in poultry farms

Out of 40 farms visited, 20 were found to report the use of antihelmentic for deworming chickens without significant differences between production systems, although a numerical increase with intensification of the farming system was clearly visible (Fig. 8).

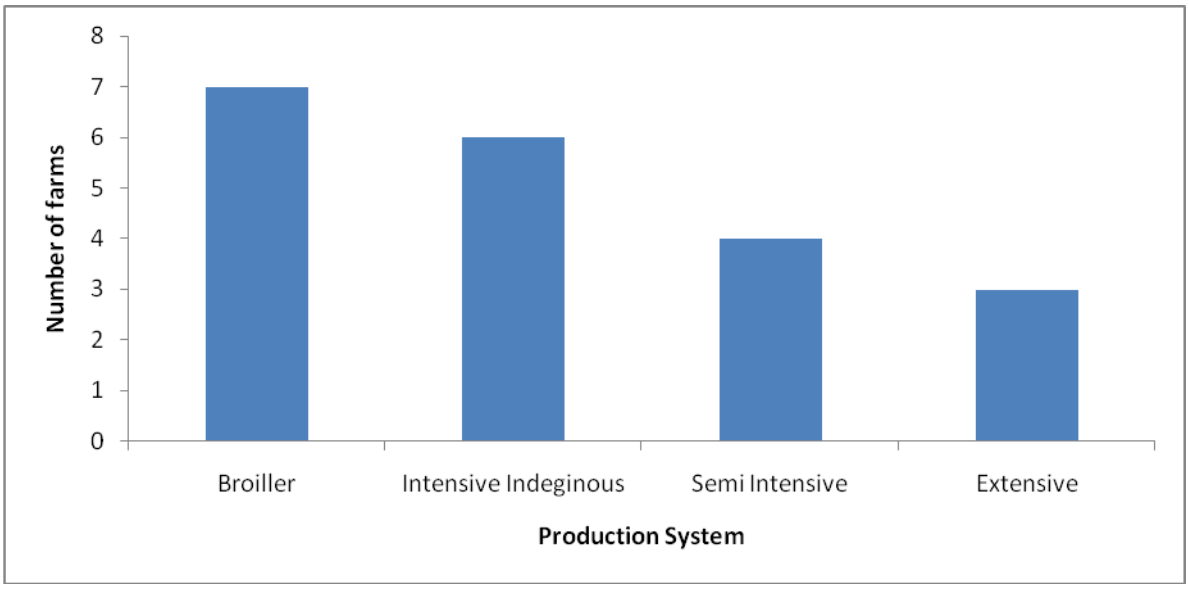

Figure 8: Number of farms that report the use antihelmentic per production system

It was observed that the proportion of farms that reported the use of antimicrobials by mixing in drinking water was numerically different between production systems, with high proportion of users observed in broiler farms. By contrast, reported antimicrobial use through feed was not different between the four production systems, although more users were observed in broiler farms. Knowledge of farmers on feeds containing antimicrobials was more commonly observed in broiler farms and was absent in extensive farms and the differences between farm types were not numerically significant (Fig. 9).

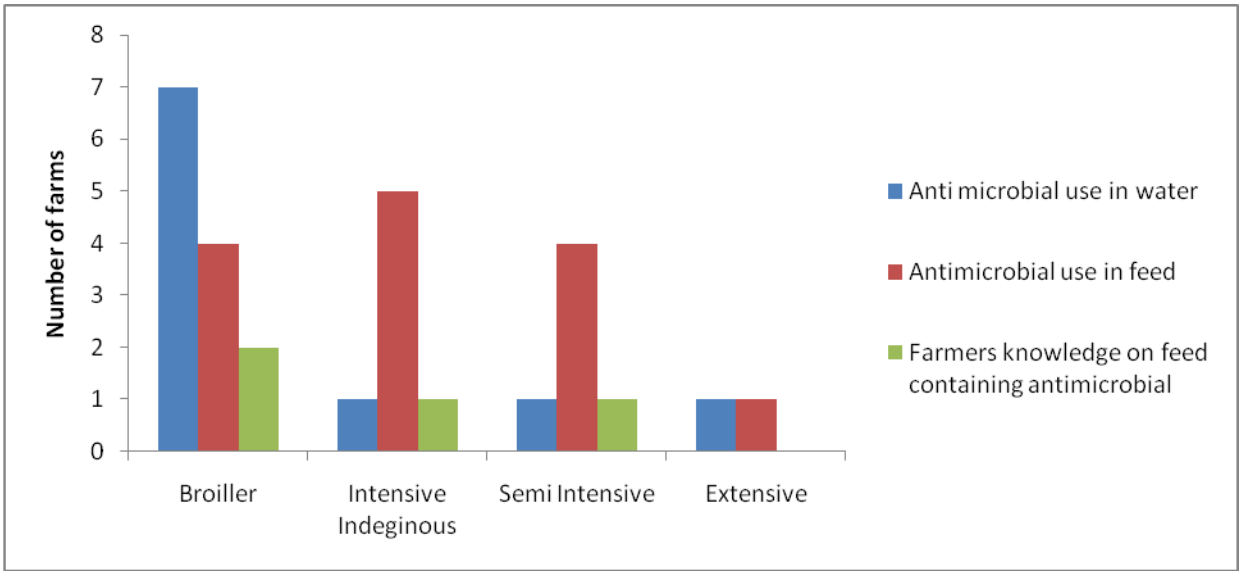

Figure 9: Antimicrobial use and farmers' knowledge on presence of antimicrobials in feed

Although the use of antibiotics and compliance with withdrawal times was high in broiler farms the difference was not statistically significant (p >0.1) (Table 3).

Table 3: Health management on poultry farms in Arusha district, Tanzania (n=10 per production system type)

Variable description (Yes/No) Variable	**Production system**				Pearson Chi-square *p*-value	Pearson χ^2 value
	Intensive broiler	Intensive indigenous	Semi - intensive	Extensive indigenous		
Antimicrobials use						
Anthelmintic	7/3	6/4	4/6	3/7	0.26	4.0000
added in water	7/3	1/9	1/9	1/9	0.002	14.4000
in feed	4/6	5/5	4/6	4/6	0.96	1.1250
Used only when animal shows clinical signs	1/9	4/6	5/5	6/4	0.12	5.8330
Knowledge on feed contain antibiotics	2/8	1/9	1/9	0/10	0.53	2.2220
Abide to antimicrobial withdraw period	7/3	5/5	3/7	3/7	0.22	4.4440
Treatment conducted by following instruction	1/9	1/9	1/9	1/9	1.00	0.0000
Use of traditional herbs	1/9	2/8	0/10	5/5	0.03	8.7500
Vaccination						
Vaccination calendar	6/4	4/6	2/8	2/8	0.18	4.8350
Common vaccination						
New castle disease	10/0	5/5	7/3	8/2	0.07	6.9333
Pox	6/4	5/5	2/8	0/10	0.02	10.3700
Zoonosis						
Farm positive to *Salmonella* (chicken)	2/8	2/8	1/9	0/10	0.47	2.5143
Environmental *Salmonella* positive farms	1/9	2/8	1/9	1/9	0.88	0.6857
Farms that both env&chicken are positive to *Salmonella*	1/2	2/2	1/1	0/1	0.80	0.9159
Farm positive to *Campylobacter (chicken sample)*	2/4	7/1	4/2	2/4	0.11	6.0273
Salmonella flock prevalence (%)	4.0	2.9	1.0	0.0	0.16	5.1084
Campylobacter [1]flock prevalence (%)	5.0	13.8	5.0	9.1	0.07	6.9101

[1] Flock prevalence (%) = (Total number of positive samples/Total number of tested samples) *100

Farm type rank base on health management

Broiler farms ranked first best in health management practices while extensive farms were ranked as worst (Fig. 10; Appendix 5).

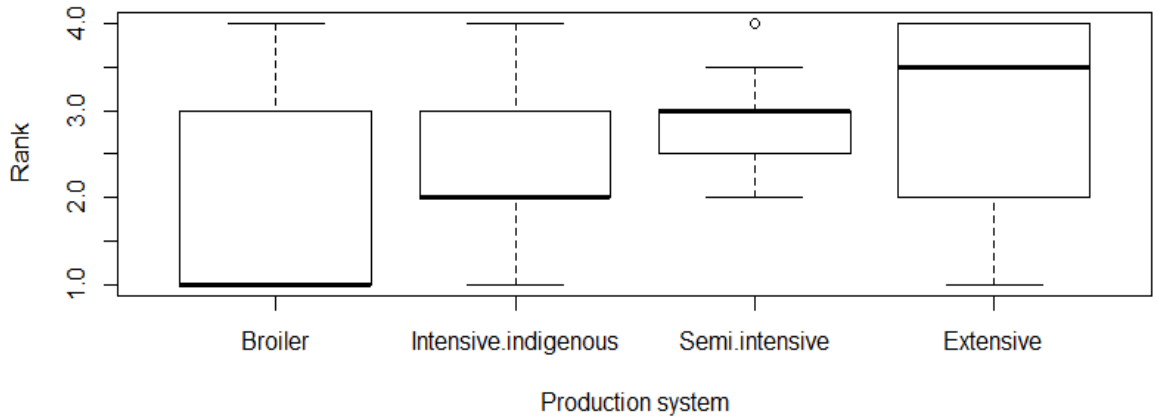

Figure 10: Farm ranking by farm type based on health management practices

Note; Broiler= "1st Best", Intensive indigenous = "2nd Best", Semi intensive = "Good", Extensive= "Worst"

4.1.6 Extension services and delivery of information and inputs to farmers

There are different extension service providers available in the area but only 8 of 40 poultry keepers are receiving service from the government. Use of government extension services decreased as intensification increased, and differences were close to significant (p = 0.10) (Table 4).

Table 4: Extension services used by poultry farmers in Arusha District, Tanzania (n=10 per production system type)

Variable description (Yes/No)	Production system				Pearson Chi-square *p*-value	Pearson χ^2 value
	Intensive broiler	Intensive indigenous	Semi – intensive indigenous	Extensive indigenous		
Farmers receiving extension services	5/5	7/3	7/3	7/3	0.72	1.319
Main extension service provider						
Government	0/10	1/9	3/7	4/6	0.10	6.2500
Input suppliers	8/2	5/5	4/6	4/6	0.23	4.3110
Non -governmental organization	0/10	1/9	0/10	0/10	0.38	3.0769
Private practitioners	2/8	2/8	3/7	2/8	0.43	0.4301
Farmers Membership group(YES/NO)	6/4	10/0	8/2	8/2	0.17	5.0000
Vaccine Source						
Extension officer	0/10	0/10	1/9	0/10	0.38	3.0770
Input suppliers	10/0	10/0	9/1	9/1	0.55	2.1053
Information Sources						
Input suppliers	1/9	1/9	2/8	1/9	0.88	0.6857
Social media	1/9	1/9	2/8	1/9	0.88	0.6857
Farmers field schools	1/9	1/9	3/7	3/7	0.60	1.8477
Colleagues	7/3	7/3	3/7	5/5	0.22	4.4444
Who finance training						
Farmers own Sources	5/5	4/6	5/5	0/10	0.06	7.4725
Government	1/9	1/9	2/7	5/5	0.71	1.3793
NGO'S	2/8	2/8	1/9	1/9	0.85	0.7843
Other sources	2/8	3/7	2/8	4/6	0.71	1.3793
Quality of extension service						
Not satisfy	3/7	3/7	4/6	2/8	0.81	0.9524
Satisfactory	7/3	7/3	6/4	8/5	0.81	0.9524
Kind of group belong						
Saving& Credit	2/8	2/8	6/4	0/10	0.02	10.133
Poultry keepers Association	7/3	7/3	3/7	0/10	0.003	14.22
Farmer field school	1/9	1/9	1/9	10/0	0.0000	27.692
Type of farmers group like to Join						
Farmer fora	3/7	3/7	5/5	5/5	0.59	1.9048
Credit and serving	6/4	6/4	4/6	4/6	0.58	1.9437
FFS	0/10	0/10	1/9	0/10	0.38	3.0769
General Husbandry	1/9	1/9	0/10	0/10	0.35	3.0769
Need to join group						
Yes/No	4/6	4/6	2/8	2/8	0.66	1.600
Need to receive information on poultry keeping						
Yes/No	10/0	10/0	10/0	10/0	NaN	NaN

4.1.7 Multiple component analysis (MCA)

Multiple Components Analysis was used to show how risk factors are associated with *Campylobacter* and *Salmonella* prevalence. Rows in the graphs (Fig. 11 to 13) shows individuals and columns are variables while dotted lines specify the null hypothesis. MCA plot give an idea of what pole of the dimension the categories are actually contributing to. The contribution of the variable categories (in%) to the definition of the dimensions was extracted using R command: head (var$contrib, 2, 4) (Appendix 5). The variable categories with the larger value, contribute the most to the definition of the dimensions.Variable categories that contribute the most to firt and second dimension (Dim.1 and Dim.2) was used as most important in explaining the variability in the data set. Quality of presentation was assessed by calculating Squred cosine (Cos^2) which measure the degree of association between variables categories and a particula axis. It is evidence that variables related to biosecurity, health management and extension Service delivery were included in separate MCA plots (Appendix 6).

(i) Multiple Component Analysis for biosecurity variables

MCA based on biosecurity variables observed that variable three chicken house in one farm ("Three") and absence of wildbirds in chicken house ("WB.No") are clustered together implying that they appear together in observations. This pattern is associated with broiler farms (Fig. 11; bottom right quandrant: Appendix 6b). Variables shown close to the centre of the graph, for example *Campylobacter* negative status and *Salmonella* negative status, represent the most common situation and explain little of the observed variation. The further away from the centre a variable is shown, the more it contributes to explanation of observed variation. *Campylobacter* positive and *Salmonella* positive are in different quadrants of the graph, indicating that they are not correlated to the same variables. This means that different control strategies may be needed for the different food borne pathogens (Fig. 11).

Figure 11: Multiple component analysis (MCA) for observed biosecurity variables
Note; "Three" -Three or more chicken houses in one farm, "Salmstatus_Pos"= *Salmonella* positive, "Campystatus_Pos"= *Campylobacter* positive, "Manure ban _No"= Farm does not have dedicated place for manure storage/disposal, "Whole family"=Farm management activity done by any member of the farmily, "SickBirdSold_Yes"=Farmers sale clinically sick chicken, "WB.No"= No wild birds observed in the farm, "Deep_Liter_Yes"= Farms that use deep liter system, "Ded_boot_Yes"= Farms that use dedicated boots, "B"=Broiller farm, "E"=Extensive farm, S_INT"= Semi intensive farm, "IND"= Intensive Indiginous farm

(ii) Multiple Component Analysis for extension delivery related variables

The three extension service providers are mutually exclusive by definition, because only one of them can be the main one in each farm. The large distance between them in the MCA confirms that they don't occur in combination. The MCA also suggests that the use of private providers is associated with broiler farms and intensive farms, and with *Salmonella* presence but not with *Campylobacter* presence. The lack of poultry skills is not closely related to *Salmonella* or *Campylobacter* presence, which suggests that skills training of farmers will not necessarily improve the food safety situation (Fig. 12; Appendix 5).

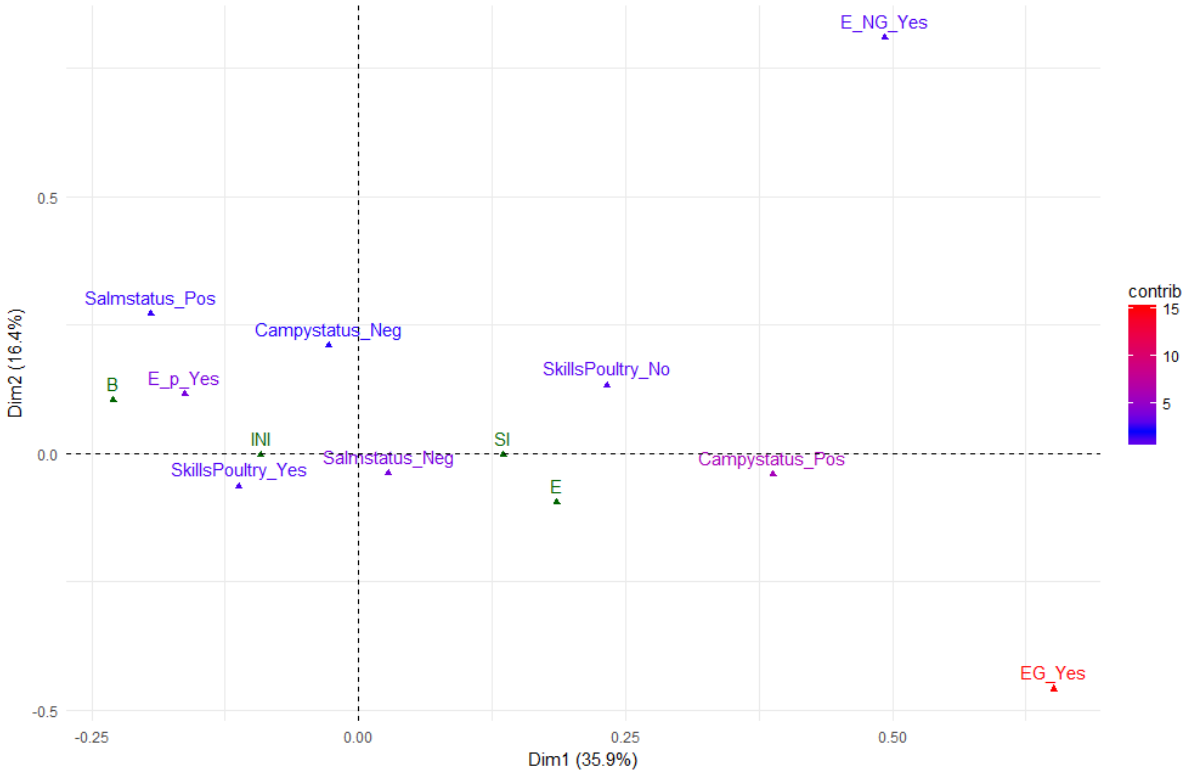

Figure 12: Multiple Correspondence Analysis (MCA) for extension service delivery related variables

Note; "EG_Yes"= main extension service provider is Government, "SkilsPoultry_Yes" = Farmers with skills in poultry production "E_NG_Yes"=Source of information was farmers field school gathering, "E_P_Yes" =main extension service provider was private sector,"B"=Broiller, "IN"=Intensive Indiginous,"SI"=Semi Intensive, "E"= Extensive**.**

(iii) Multiple Correspondence Analysis for health delivery variables

With regards to health management variables, semi-intensive and extensive farms are very similar whilst intensive and broiler farms are different. Low inputs cluster together (no antimicrobials in feed, not aware of antimicrobial withholding time but those factors are not associated with *Salmonella* or *Campylobacter* positive results. In other words, low health inputs do not imply a high food safety risk. That is important, because people might expect low inputs to lead to high risk (Fig. 13; Appendix 5 a).

Figure 13: Multiple Correspondence Analyses (MCA) for health related variables
Note;"AMWH_No"=Farmers not aware of antimicrobial withholding time, "AMWH_YES"=Farmers are aware of antimicrobial withholding time "USEVACC_Yes"=Farmers vaccinate by abiding to vaccination callender for common diseases, "AMFEED_Usually"= Farmers are usually add antimicrobial in feeds during feeding, "AMFEED_No"=Farmers do not add antimicrobial in feed, "UseVacc_No"=farms that do not follows vaccination calendar during vaccination. "Vacc_ pox_Yes"= Farmers vaccinate chicken against pox virus, "AMHU_Yes" = Farmers are aware to effect of antimicrobial to human health).

4.2 Discussion

4.2.1 Occurrence of *Campylobacter* and *Salmonella*

The overall occurrence of *Campylobacter* and *Salmonella* spp. at farm level were 15 % (6/40) and 57.7 % (15/26), based on cloacal swabs and 12.5% (5/40) for *Salmonella* based on environmental samples. The presence of *Salmonella* spp. in the environment may result from colonization of the chicken flocks and it may also contribute to colonization in chicken flocks though an oral-faecal transmission cycle, as previously described in humans and animals (Ruby *et al.*, 2012). There is a numerical trend in *Salmonella* occurrence that is not significant but suggests that further research on *Salmonella* in chicken with more sample collection may be useful. Prevalence of Campylobacter along the gradient of intensification of indigenous chicken suggested that, as farmers intensify indigenous chicken the more they are colonized with *Campylobacter* spp (Fig. 4).

Similar observations were reported in a study done in Ethiopia in which prevalence of *Campylobacter* spp in indigenous chicken was observed to increase as intensification level increases (Brena *et al.*, 2016). Therefore, it could be interesting to study specific risk factors of infection in these systems by collecting more data. Further characterization of *Campylobacter* isolates is also important. For the HAZEL project, this will be done by whole genome sequencing, which will allow comparing the chicken isolates to human isolates. Human isolates will not be obtained from Tanzania in the coming study, but access to a collection of human isolates from East Africa (CDC) Kenya. This comparison will show whether the *Campylobacter* strains that are found in chickens contribute to the public health problem.

4.2.2 Biosecurity and health management

The result from the questionnaire showed differences in biosecurity and health management of the four production systems. Farm biosecurity measures are used to control and prevent disease in poultry, these reduce but do not eliminate the risk of infection and disease (Msami, 2008). Biosecurity measures are often aimed at promoting animal health rather than human health. The biosecurity measures on poultry farms in Tanzania may protect the birds from avian disease, but they don't protect them from asymptomatic carriage of *Salmonella* and *Campylobacter*. For the chickens themselves, that is often not a problem because *Campylobacter* spp. and most *Salmonella* spp. don't cause disease in chickens. It can become

a problem for public health if the bacteria from the chickens enter the food chain. However, I was unable to link occurrence of *Campylobacter* and *Salmonella* with specific risk factors. In this study addition of antimicrobials in poultry feed are commonly used in intensive farms but poultry keepers have limited awareness that commercial feeds may contain antimicrobials too. Antimicrobial feed additives have been used worldwide in animal production for many decades because of their favorable economic effects in livestock (Stallones *et al.,* 1980; Haol *et al.,* 2014). There is an increase in public concern about the possible link between their use and transfer of antibiotic resistance organisms and genes to humans (Butaye *et al.,* 2003; Nikolay *et al.,* 2016; Zishiri and Zishiri, 2016). Antibiotic residues and resistant strains might be transmitted to humans by the consumption of poultry products which could spread to the community through the food chain (Founou *et al.,* 2016). Also occupational practices may expose people to such residues and strains through direct contact.

In this study it was observed that a good number of farmers were not abiding by antimicrobial withdrawal periods. Furthermore, chicken with disease or under treatment can be sold quickly in order to save funds and generate income or slaughtered and used as food at household level. According to regulations and guidelines on the use of veterinary drugs, antibiotics should only be employed to treat bacterial infections respecting the dose and the length of treatment and the withdrawal time provided by the manufacture or indicated by the veterinarians (Kim *et al.,* 2013). Failure to observe antibiotics withdrawal periods by poultry farmers is likely to expose consumers to products containing residues above tolerable limits (Mubito *et al.,* 2014). Also, it may create both difficulties for prevention of disease spread and food safety risks for consumers in particular, because those practices lead to the high risk of undesirable antimicrobial residues in animal products (Aarestrup and Wegener, 1999; Van Den Bogaard and Stobberingh, 2000). Antimicrobial residues may cause allergic reactions (Stallones *et al.,* 1980) and may exerts selective pressure that favors survival of resistant bacteria (Marshall and Levy, 2011), while the prudent use of antimicrobials could contribute to the lowering of the prevalence of antimicrobial resistance (Abdi *et al.,* 2017). This is in line with objective four of Tanzania National Antimicrobial Action Plan 2017-2022, which focus on optimizing the use of antimicrobial agents in human, animal and plant health (URT, 2017). Therefore, careful monitoring of the use of antimicrobial is necessary in both animals and human settings.

4.2.3 Extension service delivery

It was observed from this study that extension services in Arusha District are provided by different stake holders including government and private sectors. However, farmers who are practicing intensive production are getting most of their services from private sector providers compared to extensive farms that rely more on government extension providers. This is supported by the Tanzania livestock policy of 2006, that promote private sector to provide extension services for livelihood improvement (Mattee and Rutatora, 2001; URT, 2006). However, it may also create a risk for poultry health and public health if the advice that is provided differs between categories of extension providers. Although most farmers do not produce broilers, the number of birds produced for consumption is much higher in broiler farms than in other farm types.

Therefore, poor management of poultry health, hygiene or food safety at broiler farms poses a high potential public health risks. It is important that government policy and official advice on antimicrobial use and food safety reaches this relatively small but highly productive group of poultry farmers. Therefore the government of Tanzania should empower extension officers with transport and extension kits dedicated for livestock services, also supportive supervision should be provided to update extension officers with new technologies and approaches.

CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

Prevalence of *Campylobacter* in the study poultry farms was much higher than that of *Salmonella* spp. at farm and flock level. *Campylobacter* spp. were more prevalent in intensive indigenous when compared with other farm types (Table 3). Occurrence of *Campylobacter* and *Salmonella* pathogens were driven by different factors, which may need different control strategies.

Antimicrobials in poultry feed are commonly used in intensive farms but poultry keepers have limited awareness that commercial feeds may contain antimicrobials and associated risks.

Broiler farms were found to rank best in both biosecurity and health management practices but this does not protect chickens from the asymptomatic carriage of *Campylobacter* and *Salmonella* spp.

Despite the existing good policy which governs extension service delivery in Tanzania, private sectors were observed more involved with broiler and intensive indigenous farmers while other farm types are not having access to private sector Continuing with this approach, a double standard of service to farmers will be observed. There may be a gap in service provision by the private sector, which could be balanced by information from government extension services. As stated by one of the farmers in the participatory feedback workshop done at Arusha City council (Both Extension field officers and poultry keepers were the participants) , the private sector has a vested interest in selling their products first (Appendix 6a). This may include the sales of antimicrobials or feed. They may not want to tell farmers about the risks associated with antimicrobials. Extension field officers don't have a strong commercial incentive to provide extension service. By contrast, they are government employees and are expected to explain government policy to farmers, including the Tanzanian policy on Antimicrobial Resistance (AMR) and responsible use in poultry production.

5.2 Recommendations

The presence of *Campylobacter* and *Salmonella* in the four production system is quite possible that the source of infection is different for the two pathogens which were beyond the power or scope of the data collected. Therefore the author is recommending sampling of more chickens for test this may help to make some of the numerical differences statistically significant. Sampling of potential reservoirs, e.g. feed, would mean extending the scope of the study by including samples other than those from chickens. This may also yield new and relevant information. Furthermore, good hygienic practices during and post-slaughter handling operations of chicken are important to reduce the transfer of pathogens in the food chain. Appropriate usage of antimicrobials as per prescription should be advocated to all farm type; training of farmers on the importance of antimicrobial withholding times in relation to public health should be given consideration.

Both the private sector and Government are stakeholders in extension service provision and are required to work together to improve their service to poultry keepers. The present national policy for extension service delivery and the National Action Plan on Antimicrobial Resistance should be interpreted and practiced at the farm level. Extension services should be delivered equally in all farm types to avoid double standard of service to farmers. Biosecurity measures of poultry farms should be observed and improved in all farm types, because they may protect birds from avian diseases. Additional measures may be needed to protect birds from asymptomatic carriage of *Campylobacter* and *Salmonella* but the risk factors and protective measures need further study before specific recommendations can be made for poultry production in Tanzania.

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APPENDICES

Appendix 1: Household questionnaire

Enumerator Name:----------

	Biodata **Date…**……District……Ward……Street name----…….…… household number (if applicable):……Production system…….. ……Role of the response ……..experience of poultry keeping (in years)…….	Code
A	**Economic& Cultural information**	
1.	Age of the head of the household (Years) (01). 20 to 29, (02). 30 to 39, (03). >40	
2.	What is your Marital Status? (01). Single, (02). Married (03). Separated, (04). Widower	
3.	Type of Household (01). Father headed …….. (02). Mother Headed……	
4.	Education level Highest level of education: 1=Standard 7; 2=Form IV; 3=Form VI; 4=Diploma ; 5=University; 6=Adult education ; 7=No education	
5.	What is the occupation of the head of the house……..	
6.	What is the total number of children under 18 years of age in the household? Description gender age Numbers	

7.	How many are you in the family including yourself depend on this poultry project as a source of income?	
8	a) Main source of household income, 1= Livestock/Poultry keeping; 2=Crop production ;3=Formal employment; 4=Business; 5= Other (Specify)---------- b) How did you start keeping poultry? 1. Bought using my own money, 2. Gift from relative/friend, 2.Bank Loan, 3.Other specify……..	
9	How many dependants are depending on this project for Meat? ……	
10	a) Do you employ labour at any time of the year? Yes or No b) If yes, what type of labour do you employ on your farm? Full-time/Permanent {> 6 months}, Seasonal and Long term/Temporary {1-6 months}, Casual {On daily basis}, Other specify	
11	Who supervises farm activities? Husband, Wife, Son, Daughter, Attendant, Other specify……..	
12	Who does the following farm activities? Watering, feeding, cleaning feed and water utensils, selling eggs/ birds,----------,----------,---------- ……	
13	Who provides money to purchase poultry feeds, veterinary drugs etc?.....	

14.	Who receives money from poultry product sales? (e.g. Mother, father etc)……..	
15	Who receives money from live bird's sales?? ? (e.g. Mother, father etc)……..	
B: Extension services and management practices		
	Quality of extension services	
16	Are you receiving extension service in your area? (01). Yes, (02). No (03). I don`t know	
17	Who is the main extension service provider? 01. Government, 02. NGO, 03. Input suppliers, 4.private veterinary practitioners. 6 others	
18	How many times have you receive extension service at your farm during production cycle. 01. Once 02. When a problem arise.03. Part of my project 04. No any	
19	If never, have you ever requested for such advisory service? Yes or No	
20.	Do you have skills in poultry production? 01. Yes 02. No	
21	If yes in no 19 above where did you get the training (mention)……..type of training 1=disease control 2= feeding 3= entrepreneurship 4= marketing (others mention) ……..	
22	Rank the quality of extension service that you're getting 1=not satisfy, 02 not satisfying, 03, good it satisfy my need. 0 4. Very good, 05 excellent	
23	Who finance the training (Farmer own source; Gvt, Ngo, others – specify)	
24	Are you a member of any farmers group? 01 YES, 02. NO	
25.	What Kind of group are you belong (mention example farmer field school	

	(FFS), association platform etc)	
26	Would it be useful to you to belong to a group and, if so, what sort of group would you like?	
27	Would you like to receive information from the extension service and, if so, on which topics?	
C	**Sources of feeds, day old chicks and water**	
28.	What are the sources of water common used for feeding your chicken 1.Tape water (AUWASA) 02. Bore hole 03. River water 05. Wells 06. Others (specify)	
29.	What type of feeds common used in feeding your chicken 01.commercial 02.home made 03. Scavenge 04.both commercial and homemade 05. Commercial, homemade and scavenge.06. commercial and scavenge	
30	What are qualities of feed are you looking during procurement of animal feed (mention)	
31	Do you know whether you buy animal feed that contain antibiotics? 01. Yes 02. No	
32	01. Feed 02. Water 03. Both 04. Not at all. How often do you add antibiotics? (mention, e.g. all the time, at certain times only (specify), when animal is sick)	
33	If question 19 above is correct list types of antibiotics you have used in the past 6 months.	
34.	Mention any supplement/additive that you are adding to the feed or water for feeding your chicken----------,----------,----------	
D	**Hygiene/sanitation /Medicaments/ vaccination /mortality rates**	
35.	Do you vaccinate your chicken 1.Yes, 2 NO	
36.	Do you have a written vaccination calendar 1.Yes 2. NO	
37	What kind of vaccine is common for your farm (mention)……,…….	
38.	What are the sources of the vaccine 01. Extension officer 02. Input suppliers 03. Other (specify)	

39	Have you encounter any disease after vaccination? 01.Yes 02.NO (If yes, describe nature of the disease for each vaccine)	
40	If the answer is yes in q no 26, what is the mortalities 01) less than 5% 02) 5-10% 03) 10-20% 04) 21-50% 05. 100%	
41.	What are bases of choosing drugs 01; Experience, 02; Drug seller, 03 Veterinarian, 04; other (friends, marketing, books, and news papers etc?)	
42	What criteria do you consider when buying drugs, e.g. price, package size, ease of administration, shelf life, country of origin, other (specify)	
43	Are you using veterinary drugs in compliance with sanitary legislation? 01. YES 02.NO	
44	Which disease is a most problematic in your farm----------,----------,-----	
45	Which age group is most affected……..,…….	
46	Do you mix birds of different age groups in one pen..?	
47	Do you mix birds of different species…01. YES 02 .NO. If yes, specify (ducks, geese, turkeys)	
48	Do your chicken come in contact with other animals e.g. ducks, geese, turkeys, dogs, cats, cattle, sheep, goat, donkey, wild birds, bats, rodents indicate 01.YES, 02 .NO in the table bellow Animal Yes NO Ducks ☐ ☐ Gees ☐ ☐ Turkeys ☐ ☐ Dogs ☐ ☐ Cats ☐ ☐ Cattle ☐ ☐ Sheep ☐ ☐ Goat ☐ ☐ Donkey ☐ ☐ Wild birds ☐ ☐ Bats ☐ ☐ Rodents ☐ ☐	

49	What are the sources of daily old chick 01) local available hatchery 02) natural by using my own chicken 03) from other part of the country 04) outside the country	
50	Do you practice All in All out principle? 01.= Yes 02=No.	
51	If yes what is the gap between one butch and another (state numbers in days)	
52	Which of the following is the main source of information sources 01? Friends and colleagues 02. Media (TV, radio), 03 Drug sellers 04. others	
53	What is done with ill bird 01. Changing remedies 02. Selling quickly 03. Slaughter and consuming in family 04.destroying 05.feeding other animals	
E	**Application of antibiotics and antimicrobials and awareness of public health implications**	
54	What is the means of administering drugs 01. Through injection (IV,IM) 02.water	
55	How do you treat your chicken 1.Myself after getting advice 2.Only by following manufacture product instruction 3. I used to call a veterinarian/ Livestock field officers for treatment 4. All the above is applicable	
56	Do you treat your chicken using traditional Herbals 01, YES 02. NO	
57	If YES in question above list them here…….,…….,……	
58	At what time do you apply antimicrobials? 1. As soon as clinical symptoms appear 2. Only for prevention infectious disease (choose one or both)	
59	If you have a sick bird, how do you conduct treatment 1=individual bird, or 2=the whole group of birds	
60	Awareness of restriction of the use of certain antibiotics on poultry production 01. YES 02. NO	
62	If yes what are those…….	
63	Are you aware of the antimicrobial withdraw periods	

	01. Yes 02. .No	
64	If yes in q 40 above, are you abide them? 01. Yes 02. .No	
65	If no in q 41 above give reasons----------	
66	If yes in q 41 also give reasons…….	
67	If happen to slaughter chicken who is responsible for slaughtering and dressing the chicken? ----------,----------	
68	Who eat the animal 01. Home family 02. Sold to others (caterers, individuals, restaurants)	
69	Are you aware on the effect on human health if someone eat chicken products which has ant microbial residue? 01. No 02. YES	
70	Is your neighbor keeping chicken 01. YES 02. NO	
71	If question 44 is true how far from your farm (estimate -distance in meters, Km…….	
72	Do you provide your chicken with ant helminthes? 01. Yes, 02. No	
73	If q 48 above is yes mention them----------	
74	1. Presence of insecurity barriers (Biosecurity measures [any physical barrier, using dedicated boots, foot dips, any rodent control, manure disposal etc] write down 01. YES 02. NO Insecurity Barrier/ type used ☐ Yes ☐ No Physical barrier ☐ ☐ Using dedicated boot ☐ ☐ Foot dips ☐ ☐ Any rodents control ☐ ☐ Manure disposal ☐ ☐	

	Others (specify)	
75	a) Do you use deep liter system? 01. Yes 02. No	
	c) If yes in 'a' above, do you treat litter material before prior to new stalk? 01. YES .02 NO	

Appendix 2: Lab protocols

Title: Isolation and Identification of thermophilic *Campylobacter* spp. for HAZEL project

PURPOSE

Campylobacter is considered to be the most common bacterial cause of human gastroenteritis in the world according to the World Health Organisation (WHO, 2012). Campylobacter species can cause mild to severe diarrhoea, with loose, watery stools often followed by bloody diarrhoea. *C. jejuni, C. coli*, and *C. lari* account for more than 99% of the human isolates (FDA-BAM, 2001).

Campylobacter species are highly infective. The infective dose of *C. jejuni* ranges from 500 to 10,000 cells, depending on the strain, environmental and host conditions. Thermophilic species (optimum 42°C) such as *C. jejuni* are occasionally invasive. The infections are manifested as meningitis, pneumonia, miscarriage, and a severe form of Guillain-Barré syndrome (Nachamkin et al.,1992).

Campylobacters are carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. They can establish a temporary asymptomatic carrier state, as well as illness, in humans. This is especially prevalent in developing countries. Consumption of food and water contaminated with untreated animal or human waste accounts for 70% of Campylobacter-related illnesses each year. The foods include unpasteurized milk, meats, poultry, shellfish, fruits, and vegetables (FDA-BAM, 2001).

Environmental stresses, such as exposure to air, drying, low pH, heating, freezing, and prolonged storage, damage cells and hinder recovery to a greater degree than for most bacteria. Older and stressed organisms gradually become coccoidal and increasingly difficult to culture. Oxygen quenching agents in media such as hemin and charcoal, as well as a micro-aerobic atmosphere and pre-enrichment, can significantly improve recovery (FDA-BAM, 2001).

Campylobacters are micro-aerophilic, very small, curved, thin, Gram-negative rods (1.5-5 µm), with corkscrew motility. *Campylobacter* spp. are currently identified by tests described by Harvey (1980) and Barret et al. (1988). PCR genus and species identification methods have been published.

I. SCOPE

This standard operating procedure (SOP) details the procedures to be followed to isolate, enumerate, biochemically confirm and store thermophilic *Campylobacter* spp. from animal samples in the Zoonoses Laboratory.

II. STANDARD PRECAUTIONS

Title: Isolation and Identification of *Salmonella* spp. For HAZEL project

PURPOSE

Salmonella are motile Gram-negative facultative anaerobic bacteria in the family of Enterobacteriaceae. The *Salmonella* genus consists of two species, *Salmonella enterica* and *Salmonella bongori*. Most pathogenic species of *Salmonella* causing illness in human belong to the *Salmonella enterica* species. This species is further divided into 6 subspecies: *Salmonella enterica* subspecies enterica, salamae, arizonae, diarizonae, houtenae and indica (Grimont and Weill, 2009).

Salmonella live in the intestinal tract of a host. Some *Salmonella* serotypes are host-specific while some have a more generic host range. Serotypes that cause no symptoms in animal can result in infection in humans, and vice versa (USDA, 2011).

Globally, a large percentage of salmonellosis cases are foodborne (Majowicz et al., 2010). Yet, other routes of transmission such as contact with pets or pet foods, direct personal contact, nosocomial transmission, waterborne transmission, and contaminated drugs, are also important (Hoelzer et al 2011).

I. SCOPE

This standard operating procedure (SOP) details the procedures to be followed to isolate, enumerate, biochemically confirm and store *Salmonella* spp. from animal samples in the Zoonoses Laboratory.

II. STANDARD PRECAUTIONS

Basic laboratory safe working practices should be followed at all times and all individuals conducting the activities described should first review, understand and sign the general KCRI Safety SOPs (SAF 001-007; EQP003; EQP007; EQP008; EQP011; BIO010 and POL003.01). Lab coat and gloves should be worn to perform all of the preparation activities described in this SOP.

The equipment used for these procedures should be clearly marked “HAZEL project”.

III. METHOD AND SOURCE

Modification of FDA-BAM Salmonella

- Equipment and Materials
- Incubator 37 ± 2°C
- Incubator 42 ± 2°C
- Filter stomacher bags
- Sterile P30’s
- Sterile loops 10 µL
- Sterile loops 1 µL
- Pipette 20 - 200 µL
- Sterile pipette tips 200 µL

- Stomacher
- Vortex
- Spiral Plater
- Microscope
- Microscope slides
- Cryovials

Media/Reagents

- Buffered Peptone water (BPW)
- Rappaport-Vassiliadis (RVS) Broth (in 10ml quantities)
- Xylose lysine deoxycholate (XLD+N) agar with Novobiocin
- MacConkey (MAC) agar
- Tryptic Soy agar (TSA)
- Columbia Blood Agar (CBA)
- Tryptone broth (for Indole test)
- Kovac's reagent
- Urea slopes/Rapid Urea tests
- Lysine Iron (LIA) agar slopes
- Triple Sugar Iron (TSI) agar slopes
- Phosphate Buffer Saline (PBS)
- Maximum Recovery Diluent (MRD)
- Oxidase test strips
- Catalase (H_2O_2)
- Gram stain reagents
- Microbact 12A/API20E biochemical test strips
- Salmonella Poly O antisera
- Salmonella Poly H antisera
- Glycerol
- Brain Heart Infusion (BHI)

The media listed above are available commercially. Directions for preparation, as given by the manufacturer, should be followed.

Quality Control

Positive Control Culture	- *Salmonella* spp. (non-Typhi) from KCRI
Negative Control Culture	- *Escherichia coli* from KCRI

Positive and negative controls as well as a sterility control should be set up alongside samples each time they are set up and followed through the method to ensure all steps are working effectively.

IV. SAMPLE ENRICHMENT PROCEDURE

Raw and cooked Meat Products

1. Label filter stomacher bag with appropriate sample number (i.e. M001).
2. Using sterile utensils, aseptically weigh a 25 ± 2 g sample into a filter stomacher bag.
3. Add 225 ml of BPW.

4. Mix the sample thoroughly in a stomacher for 30 s. Alternatively fold the top of the bag over several times and massage the sample in the bag vigorously but carefully for approximately 30 s.

5. Retain a small portion of pre-enriched sample for enumeration purposes (or other method).
6. Incubate at 37 ± 2 °C for 18-20 h.
7. Subculture 0.1 ml of enriched broth into 10 ml RVS broth.
8. Incubate at 42 ± 2 °C for 24 h.
9. Plate a 10 ul loopful of RVS enrichment onto an XLD+N plate. Streak for isolation using a sterile 10 µL loops
10. Incubate at 37 ± 2 °C overnight.
11. Proceed to isolate identification/confirmation.
12. Retain the enrichment broth until sample has been processed.

Environmental swabs

1. Label the tube or stomacher bag with appropriate sample number (i.e. E001)

2a. For an Amies swab, aseptically cut cotton tip end of swab into plastic Universal tube containing approximately 20 ml BPW.

2b. For a polyurethane swab (pre-moistened with 10 mL of diluent) in a stomacher bag, add 90 ml of BPW.

3. Either vortex the tube (1a) or mix the sample thoroughly in a stomacher for 30s (1b). Alternatively fold the top of the bag over several times and massage the swab in the bag vigorously but carefully for approximately 30 s.
4. Retain a small portion (at least 3 mL) of pre-enriched sample for enumeration purposes (or other method).
5. Incubate at 37 ± 2 °C for 18-20 h.
6. Subculture 0.1 ml of enriched broth into 10 ml RVS broth.
7. Incubate at 42 ± 2 °C for 24 h.
8. Plate a 10 uL loopful of RVS enrichment onto an XLD+N plate. Streak for isolation.
9. Incubate at 37 ± 2 °C overnight.
10. Proceed to identification/confirmation steps.
11. Retain the enrichment broth until sample has been processed.

Faecal Samples

1. Label the tube or stomacher bag with appropriate sample number (i.e. F001)
2. Using sterile utensils (i.e. a disposable loop), weigh 0.5 ± 0.2 g of faeces (approximately a pea sized faeces sample) into 20 mL of BPW in a sterile container with leak-proof lid (e.g. a P30 vial).
3. Vortex the tube for 10 s.
4. Retain a small portion of pre-enriched sample for enumeration purposes (or other method).
5. Incubate at 37 ± 2 °C for 18-20 h.
6. Subculture 0.1 ml of enriched broth into 10 ml RVS broth.
7. Incubate at 42 ± 2 °C for 24 h.

8. Plate a 10 uL loopful of RVS enrichment onto an XLD+N plate. Streak for isolation.
9. Incubate at 37 ± 2 °C overnight.
10. Proceed to identification/confirmation steps.
11. Retain the enrichment broth until sample has been processed.

Carcass swab samples (Cattle, Sheep, Goats)

N.B. Each carcass will have two carcass swab samples to test for *Salmonella*.

1. Label the tubes with appropriate carcass sample numbers (i.e. C001a and C001b).
2. Add 20 ml BPW to the carcass swab tubes.
3. Vortex the tube for 10 s.
4 Retain a small portion of pre-enriched sample for enumeration purposes (or other method).
5. Incubate at 37 ± 2 °C for 18-20 h.
6. Subculture 0.1 ml of enriched broth into 10 ml RVS broth.
7. Incubate at 42 ± 2 °C for 24 h.
8. Plate a 10 uL loopful of RVS enrichment onto an XLD+N plate. Streak for isolation.
10. Incubate at 37 ± 2 °C overnight.
11. Proceed to identification/confirmation steps.
12. Retain the enrichment broth until sample has been processed.

Chicken carcass samples

1. Label large stomacher bag and 50-60ml tube with appropriate sample number (i.e. C001)
2. Place the whole carcass in a large stomacher bag. Add 200 ml BPW.
3. Massage the carcass gently for approximately 2 mins.
4 Retain a small portion of pre-enriched sample for enumeration purposes (or other method).
5. Pour 50 ml of the rinsate into a sterile tube or stomacher bag for *Salmonella* enrichment.
6. Incubate at 37 ± 2 °C for 18-20 h.
7. Subculture 0.1 ml of enriched broth into 10 ml RVS broth.
8. Incubate at 42 ± 2 °C for 24 h.
9. Plate a 10 uL loopful of RVS enrichment onto an XLD+N plate. Streak for isolation.
10. Incubate at 37 ± 2 °C overnight.
11. Proceed to identification/confirmation steps.
12. Retain the enrichment broth until sample has been processed.

V. ENUMERATION OF SALMONELLA SPECIES

Raw and cooked Meat Products and Environmental swabs

1. Using the pre-enriched sample retained from previous step, use the spiral plater (50 µL setting) to plate sample in duplicate onto XLD+N plates. Allow plates to dry.
2. Place plates lid-down and incubate at 37 ± 2 °C for 24 h.
3. Proceed to enumeration calculation and confirmation.

Faecal samples

1. Using the pre-enriched sample retained from previous step, make a 1/1000 serial dilution (for chicken faecal samples) using 9ml volumes of MRD. Use the spiral plater (50 µL setting) to plate these two dilutions, each in duplicate, onto XLD+N plates. Allow plates to dry.

2. Place plates lid-down and incubate at 37 ± 2 °C for 24 h.
3. Proceed to enumeration calculation and identification/confirmation steps.

Enumeration calculation

1. Count the number of typical *Salmonella* colonies using the spiral plater grid plates provide with the Whitley Spiral Plater. *Salmonella* on XLD+N appear as red colonies with or without black (H_2S) centres. Record counts and grid used.
2. Retain positive enumeration plates at 5 ± 3 °C until presence/absence tests are complete.
3. Proceed to Confirmation steps.
4. Calculate *Salmonella* numbers based on the percentage of isolates confirmed positive.

(For example if your count was 100 and 3/5 were confirmed *Salmonella*, the final count is adjusted to 100 x 3/5 = 60).

VI CONFIRMATION OF SALMONELLA SPECIES

Identification

1. Examine XLD+N plates for typical *Salmonella* colonies. *Salmonella* on XLD+N appear as red colonies with or without black (H_2S) centres.
2. Subculture at least 2 colonies onto MAC agar
3. Incubate at 37 ± 2 °C overnight
4. Discard Lactose fermenters. If colonies are non-lactose fermenters (NLF), subculture individual colonies from each of the selected presumptive Salmonella subcultures into 5 ml tryptone broth.
5. Incubate at 37 ± 2 °C for 4-24 h.
6. Inoculate TSI and LIA slopes and with the growth from the tryptone broth. Plate on TSA, CBA or MAC to ensure purity.
7. Incubate at 37 ± 2 °C overnight.
8. Add a few drops of Kovac's reagent to the Tryptone broth to test for indole production
9. Check results against Table 1.
10. Presumptive Salmonella isolates can be further confirmed using Poly H and Poly O agglutination tests as well as Microbact 12A test strips.

Confirmation test results

Indole production: To test for indole production, add a few drops of Kovac's reagent to the Tryptone broth tube. A positive test will produce a bright pink colour on the top of the liquid.

Alternatively a spot indole test may be used. Moisten filter paper with a drop of Kovak's reagent. Rub a 1 µl loopful of growth from CBA plates onto the moistened filter paper. If the reagent turns red/brown rapidly, it is a positive reaction. *Salmonella* spp. are indole negative. *E. coli* is indole positive.

TSI: Using the tryptone broth, inoculate the TSI slope using a loop. Smear the slope with inoculum and stab the butt of the agar slope. *Salmonella* spp. have an acidic reaction in the butt (yellow colour change) and gas is produced. The slope is alkaline (red). *Salmonella* spp. are usually H_2S positive. See Table 1.

LIA: Using the tryptone broth, inoculate the LIA slope using a loop. Smear the slope with inoculum and stab the butt of the agar slope. *Salmonella* spp. have an alkaline reaction in the butt (purple colour). The slope remains purple (alkaline). *Salmonella* spp. are usually H_2S positive on LIA. See Table 1.

All cultures that give an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential Salmonella isolates and submitted for biochemical and serological tests.
Microbact 12A/API20E: Follow manufacturer's instructions.

Poly O/H: Follow manufacturer's instructions.

Extra confirmation tests:

Urease: Inoculate a urease slope or urease broth. Incubate at 37 ± 2 °C for 4-24 h Positive urease reactions turn slopes a pink colour. *Salmonella* spp. are urease negative. Rapid urease tests can be used to discount some isolates (i.e. *Proteus* spp.) which show a urease positive reaction within a few hours). Urease test can be set up at the same time as the original tryptone broth to save time and media on urease positive samples.

Gram Stain: *Salmonella* spp. are Gram negative rods.

If the isolates are identified as *Salmonella* spp., grow an isolate up in BHI over night, label appropriately and freeze in a cryovial at -80 °C with 15% glycerol for further testing.

Table 1: TSI and LIA reactions (from "Salmonellae in Foods and Feeds")

	TSI REACTIONS						
LIA REACTIONS	K/A	K/Ag	K/A H2S+	K/Ag H2S+	A/A	A/Ag	A/Ag H2S+
K/K or N	Serratia (S. typhi) (Hafnia alvei)	Hafnia alvei Klebsiella (Serratia)	(Salmonella)		Serratia	Klebsiella E. aerogenes/ liquifaciens E. coli	
K/K or N H2S+	(S. typhi)	(Salmonella) (Arizona)	S. typhi (H2S+) (Salmonella) (Arizona) (Edwardsiella)	Salmonella Arizona Edwardsiella			Arizona (Salmonella)
K/A	E. coli (A-D) Shigella Morganella morganii E. agglomerans Y. pseudotuberculosis	E. agglomerans E. coli Morganella morganii Paratyphi A (S. flexneri 6) C. diversus			E. coli E. agglomerans Y. enterocolitica	E. sakazakii E. agglomerans C. diversus (E. coli) (Citrobacter)	
K/A H2S+				Citrobacter			Citrobacter
R/A	Providencia rettgeri Providencia Morganella morganii	Providencia Morganella morganii		Proteus mirabilis (Proteus vulgaris)	Providencia rettgeri		Proteus vulgaris (Proteus mirabilis)

Key:
( ) = not the most common reaction
R = red, oxidation deamination of lysine
K = alkaline slant /K = alkaline butt
A = acid slant /A = acid butt
Ag = acid and gas
H2S = hydrogen sulfide production

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Name	Signature and Date	
Author/Date:	Kate Thomas Post Doctoral Fellow HAZEL project	March 2014
Revised by:	Kate Thomas	June 2016
Approved by:	HAZEL PI: John Crump	_____

Declaration

I have read & understand the Zoonoses Laboratory – Isolation and Identification of *Salmonella* spp., and I agree to abide by the procedures described.

Name	Signature and Date
……	…….
……	…….
……	…….
……	…….
……	…….
……	…….
……	…….

Appendix 3: Ethical clearance

THE UNITED REPUBLIC OF TANZANIA

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Ministry of Health, Community
Development Gender, Elderly & Children
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NIMR/HQ/R.8c/Vol. II /653

27th September 2016

Prof. Blandina T Mmbaga
Kilimanjaro Clinical Research Institute (KCRI)
P O Box 2236, MOSHI
Kilimanjaro

APPROVAL FOR EXTENSION OF ETHICAL CLEARANCE

This letter is to confirm that your application for extension on the already approved proposal: Zoonoses and Livestock Systems Research (ZELS), (Mmbaga B *et al)*, has been granted approval to be conducted in Tanzania.

The extension approval is based on the progress report dated 09th July 2016, on the project, Ref. NIMR/HQ/R.8a/Vol. IX/2028, dated 07th October 2015. Extension approval is valid until 06th October 2017.

The Principal Investigator must ensure that other conditions of approval remain as per ethical clearance letter. The PI should ensure that progress and final reports are submitted in a timely manner.

Name: Dr Mwelecele N Malecela

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY &CHILDREN

CC: RMO
DED
DMO

Appendix 4: Farm ranking

Farm ranking based on biosecurity variables

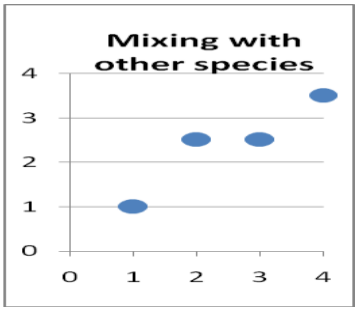
Table 5: Farm rank based on Individual biosecurity variable

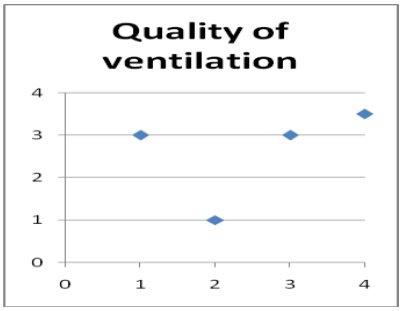
	Farm type	Mixing with different age	Mixing with other species	Contact with wild bird	Contact with rodents	Physical barrier	Dedicatetd boots	Foot dip	Rodent control	Deep litter	Quality of ventilation	All in All out	Manure diposal (Distance >20M)	Average rank	Min
Broiler	Broiler	1	1	3	1	1.5	1	1	1.5	1	3	1	1	1.41667	1
Intensive	Intensive	2	2.5	1	3	1.5	2	3	1.5	2	1	3	3.5	2.16667	1
Semi-intensive	Semi-intensive	3.5	2.5	3	3	3	3.5	3	3.5	3	3	3	2.5	3.04167	2.5
Extensive	Extensive	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	2.5	3.41667	2.5

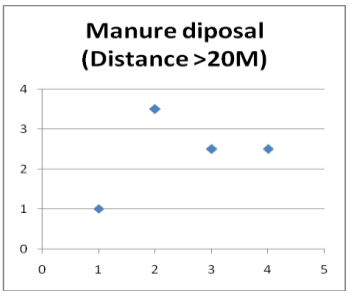
1=Best, 2= 2nd best, 3= 3rd best, 4= worst

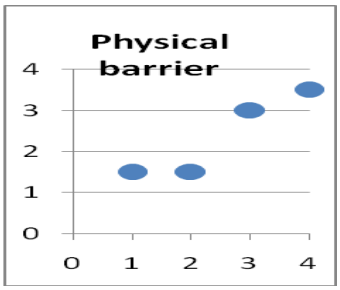
Procedure

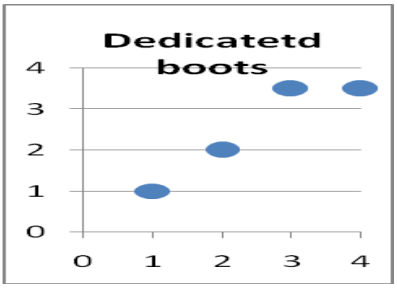
If two or three farms have the same rank the rank were averaged example if 2nd and the 3rd were the same their position were averaged(2+3/2)= 2.5. I one was better than the rest and the rest were all the same 3 was given as the number(Average of rank 2,3 and 4=3) example for foot deep

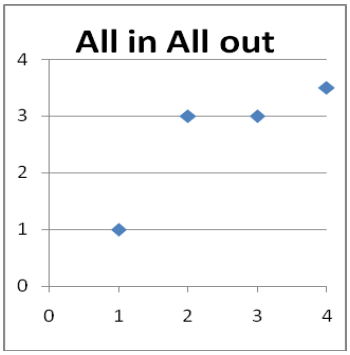


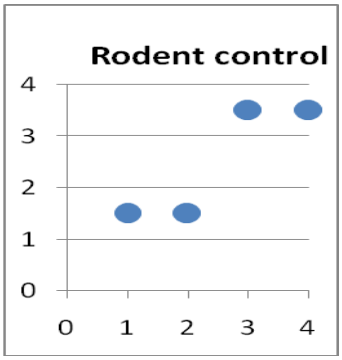


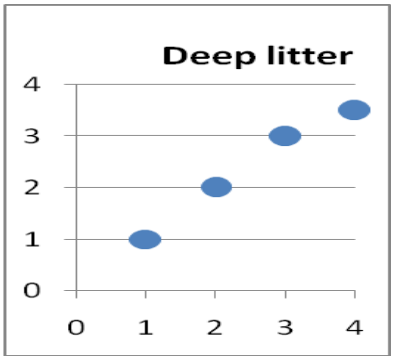


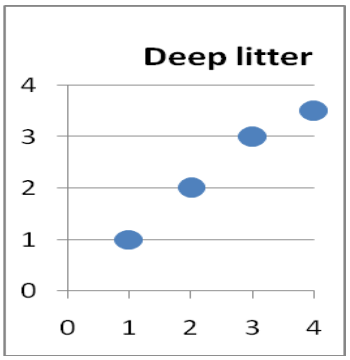












NB: The y –axis represent Farm rank (1=Best, 2= 2nd best, 3= 3rd best, 4= worst)

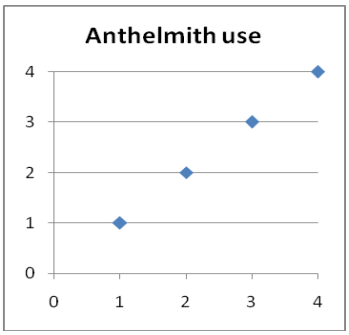
and X-axis represent production system (1=Broiler, 2=Intensive Indiginous,3=Semi Intensive and 4=Extensive)

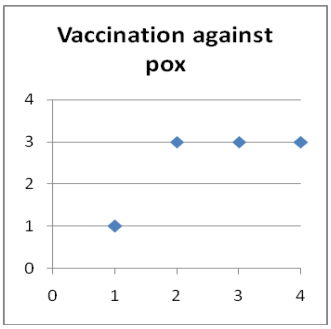
b) Health management variables

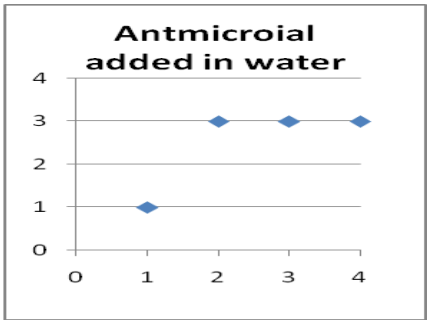
Table 6: Farm rank based on individual health management variable

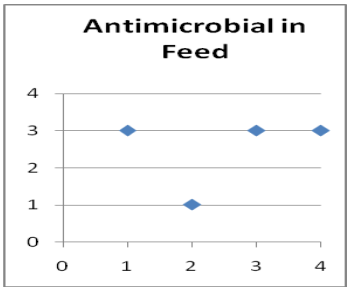
Farm type	Anthelmith use	Antmicroial in water	Antimicrobial in Feed	Antimicrobial given when animal is sick	Vacc calender	vaccinate New castle disease	Vaccinate pox virus	source of vaciines	Inpurt surpliers as source of vaccine	Follw withdraw	sale sick	slaughter sick	Destroy	treatment by folows instruction	*salmonella*	*Salmonella* present	***Campylobacter* present**	Q1	MIN	MAX	MEDIAN	Q3
Broiler	1	1	3	4	1	1	1	3	1	1	1	4	3.5	1	1.5	2.5	3.5	1	1	4	1	3
Intensive	2	3	1	3	2.5	2	3	2	2	4	2	3	3.5	2	1.5	1	1	2	1	4	2	3
Semi-intensive	3	3	3	2	2.5	3.5	3	4	3.5	3	3	2	2	3	3.5	2.5	2	2.5	2	4	3	3
Extensive	4	3	3	1	4	3.5	3	1	3.5	2	4	1	1	4	3.5	4	3.5	2	1	4	3.5	4

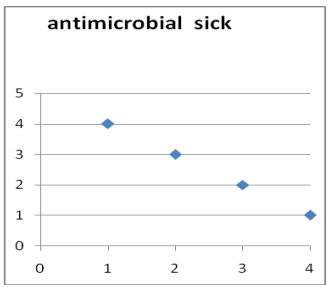
Ranking of individual biosecurity variables pre production system

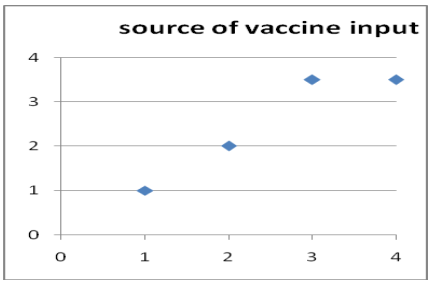


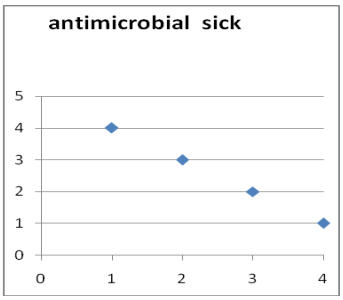


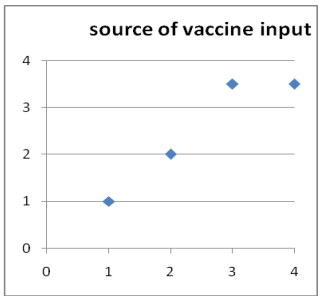


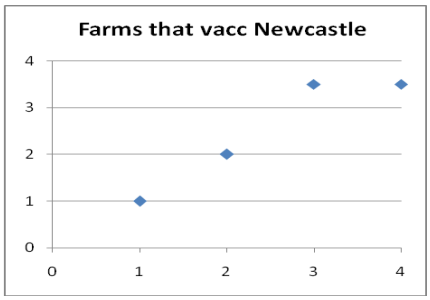


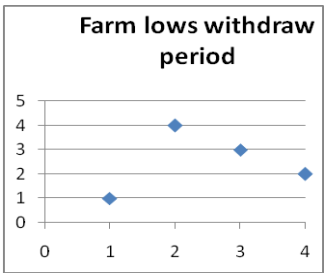


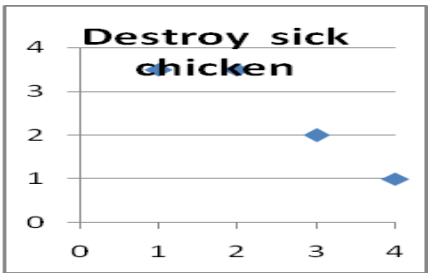


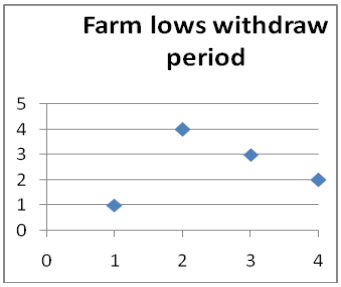


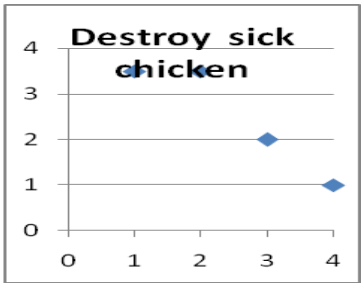


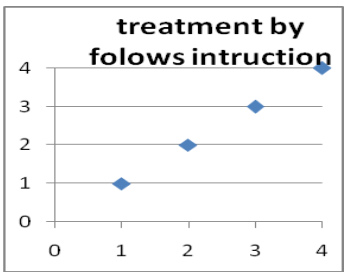


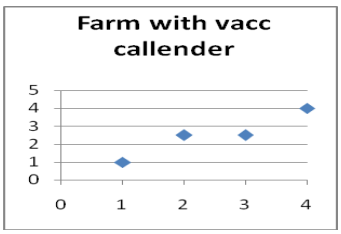


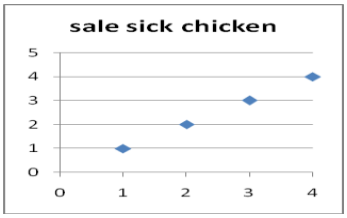


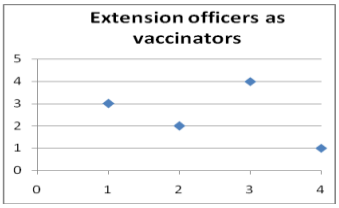












NB: NB: The y –axis represent Farm rank (1=Best, 2= 2nd best, 3= 3rd best, 4= worst)

and X-axis represent production system (1=Broiler,2=Intensive Indiginous,3=Semi Intensive and 4=Extensive)

Appendix 5: Multiple component analysis

Procedure:

- Identification of the variables that are most correlated with each dimension
- Maximum number of possible MCA dimensions was calculated from the difference between the sum of variables categories and the number of variables. Total inertia was obtained by dividing maximum number of MCA dimensions divide by the number of variables. Method used to exploring the number of dimension to be included in the analysis and to obtain the reference value for total inertia. Main use of inertia is as indicator of the number of axes to retain for further analysis. To define the number of dimension to retain the following criteria were employed (i)screen plot and (ii)Eigen value: Two dimension was selected as best allows for data interpretation
- Describing category with a contribution larger than the threshold which will be considered as important in contributing to that dimension
- All variables were first plotted, followed with plots which have reduced number of categories for easy viewing.
- With reduced categories color was used according to their contribution to the variance.
- Production system was indicated as supplementary variables
- Proportional variance related by the different dimensions (axes) were extracted
- A two dimension MCA solution was considered the most adequate
- Then plot to Identify variables that are most correlated with each dimension was done

a) MCA for health management related variables

Only 13 (Table A1) variables for health management from table 3 were included for analysis, 4 variables (Environmental *Salmonella* positive farm, Farms that both Environment and Chicken are positive to *Salmonella*, *Salmonella* flock prevalence, *Campylobacter* flock prevalence) were excluded because consideration is based on farm level. The 13 variables were reduced to 10 (Fig A1 1) based on which contribute most to dimension 1 and 2 in the MCA. Production system was considered as supplementary variable (was not used for the determination of the principal dimension). Variable of interest were production systems (Broilers, Intensive indigenous, Semi intensive and Extensive) and farm status in terms of pathogens (*Campylobacter* and *Salmonella*) were included too.

Table A 1: Variables included In MCA from table 3

NO	VARIABLES	CODE IN MCA	NUMBER OF LEVELS
1	Antimicrobial in feed	"AMFEED"	2
2	Vaccination Calendar	"USEVACC"	2
3	Abide to antimicrobial withdraw period	"AMWH"	2
4	Knowledge on effect of antimicrobial to human	"AMHUM"	2
5	Use of traditional herbs	"UseTradherbs"	2
6	Antihelmentic	"USE ANTHEL"	2
7	added in water	"AMWATER"	2
8	in feed	"AMFEED"	3
9	New castle disease	"Vacc_Gombr"	2
10	Pox	"Vac_Pox"	2
11	Farm positive to *Salmonella*	"*Salmonella* Status"	2
12	Farm positive to *Campylobacter*	"Campy status"	2
13	Treatment conducted by following instruction	"Treat_After_Instr"	2

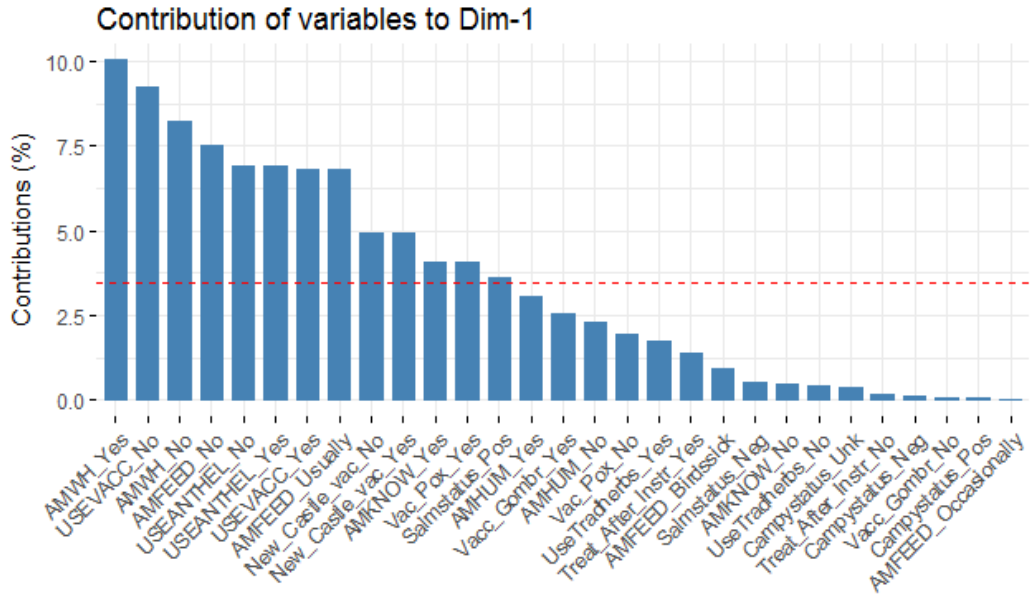
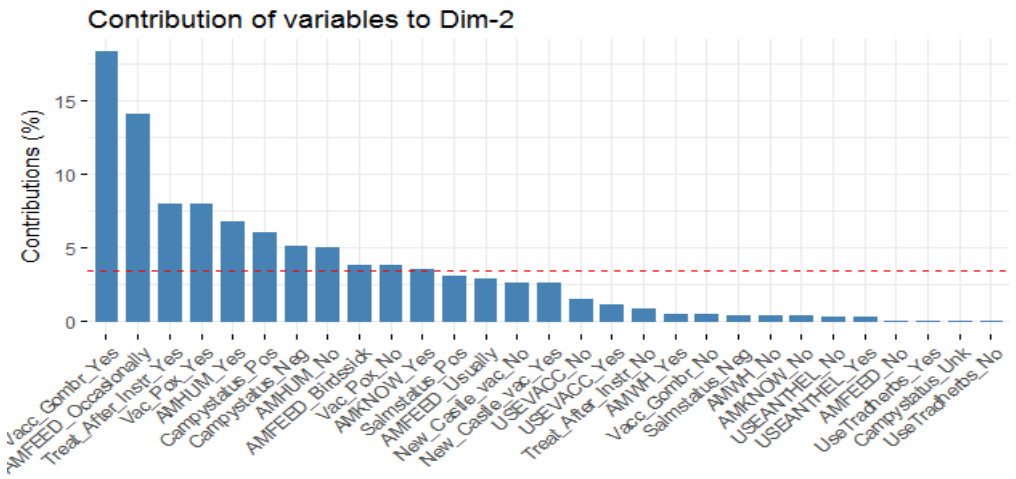


Figure A1 2 : Variables contributing to Dimension 1, bars above red dotted line are contributing most to this dimension

R command: fviz_contrib(dathea.mca, choice = "var", axes = 1)



FigureA 1 3: Variables contributing to Dimension 2, bars above red dotted line are contributing most to this dimension

R- Command: fviz_contrib (dathea.mca, choice = "var", axes = 2)

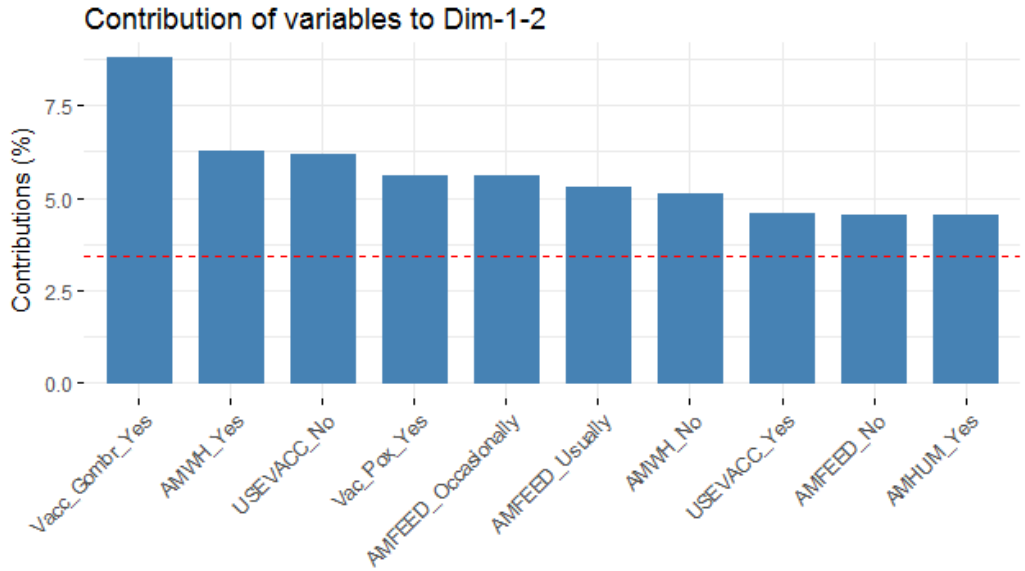


Figure A1 4: 10 variables that contributes most to dimension 1and 2

R- Command: fviz_contrib (dathea.mca, choice = "var", axes = 1:2, top = 10)

dathea.mca <- MCA (dathea, method = "burt", quali.sup=1:1)

Table A 2: Eingenvalues for selected health related variables in each dimension

Dimension	eigenvalue	Variance. Percent	cummulative.variance.percent
Dim.1	0.0484	33.4952	33.4952
Dim.2	0.0317	21.9177	55.4129
Dim.3	0.0137	9.4840	64.8969
Dim.4	0.0123	8.4994	73.3963
Dim.5	0.0093	6.4117	79.8080
Dim.6	0.0072	5.0148	84.8228

R-command

> eigenvalues <- get_eigenvalue(dathea.mca)

> head (round (eigenvalues, 4))

Figure A1 5: MCA Plot with some of the categories dropped for easy viewing

R command:

```
fviz_mca_var(dathea.mca, col.var="contrib", select.var = list(name = c("FEED_Ocasionally",
"Vacc_Gomborow_Yes", "Salmstatus_Pos", "Salmstatus_Neg","Broiler", "Extensive",
"SemiIntensive","IntIndigenous","USE VACC_No", "Vac_Pox_Yes","AMWH_Yes",
"AMFEED_Usually","AMWH_No", "USEVACC_Yes","AMFEED_No","AMHUM_Yes"
)), title = "") + scale_color_gradient2(low="white", mid="blue", high="red",
midpoint=2)+theme minimal
```

b) MCA FOR BIOSECURITY VARIABLES

All variables (Table A3) for biosecurity from table 2 were included for analysis, 4 variables. Variables were reduced to 10 based on which contribute most to dimension 1 and 2 of MCA. Production system was considered as supplementary variable (was not used for the determination of the principal dimension). Variable of interest were production systems (Broiler, Intensive indigenous, Semi intensive and Extensive) and farm status in terms of pathogens (*Campylobacter* and *Salmonella*) were also included.

Table A 3 Variables included in MCA from table 2

NO	VARIABLES	CODE IN MCA	NUMBER OF LEVELS
1	different age	"Mix_Age"	2
2	other species	"Mix_SPP"	2
3	wild birds	"W_B"	2
4	physical	"Phy_sical_barier_presence"	2
5	dedicated boots	"Ded_boot"	2
6	barrier foot dip	"Foot.dip"	2
7	Rodents	"Barier_Rod"	2
8	Use of deep litter system	"Deep_Liter"	2
9	Sale chicken with clinical signs	"SickBirdSold"	2
10	Slaughter	"SickBirdHomeKill"	2
11	Destroy un recovered chicken after treatments	"SickBirdDestroyed"	2
12	Employ Labor at any time	"Labor_type"	2
13	Whole family	"who_super_vice"	3
14	Production system	"Prod_system"	4
15	*Campylobacter* farm status	"Campystatus"	2
16	Number of chicken houses	"CH_HOS"	3
17	Presence of manure Ban	"Manure_Ban"	2

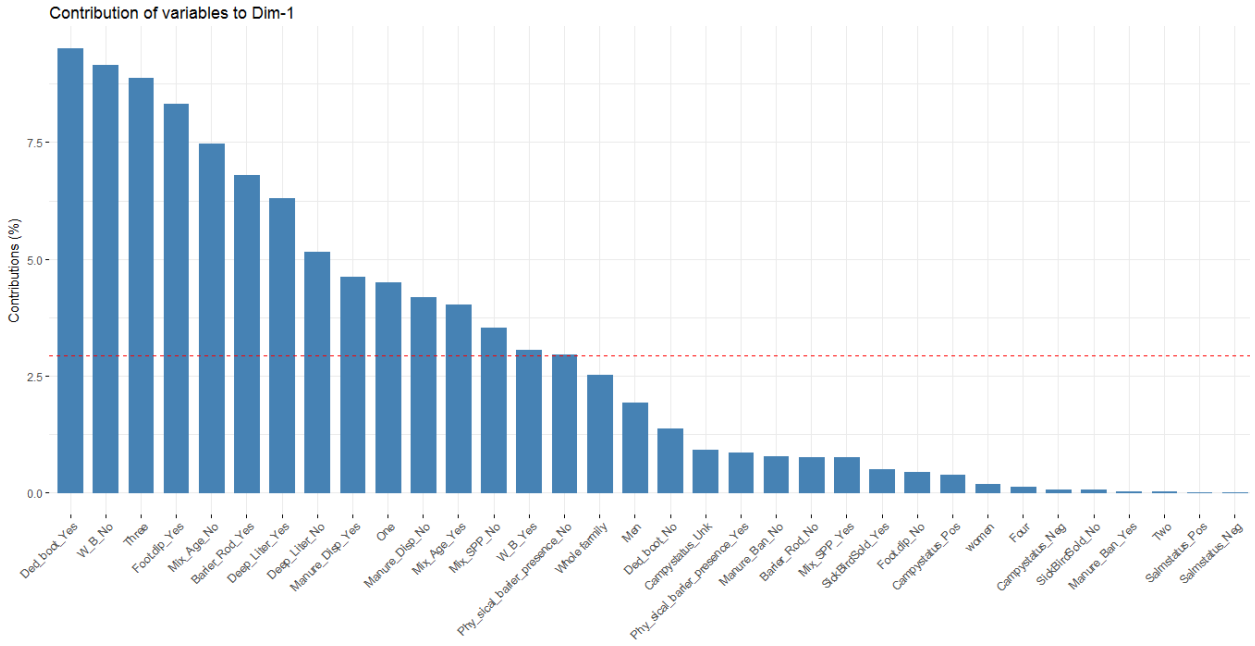


Figure A1 5: contributing to Dimension 1, bars above red dotted line are contributing most to this dimension

fviz_contrib (dBIO.mca, choice = "var", axes = 1)

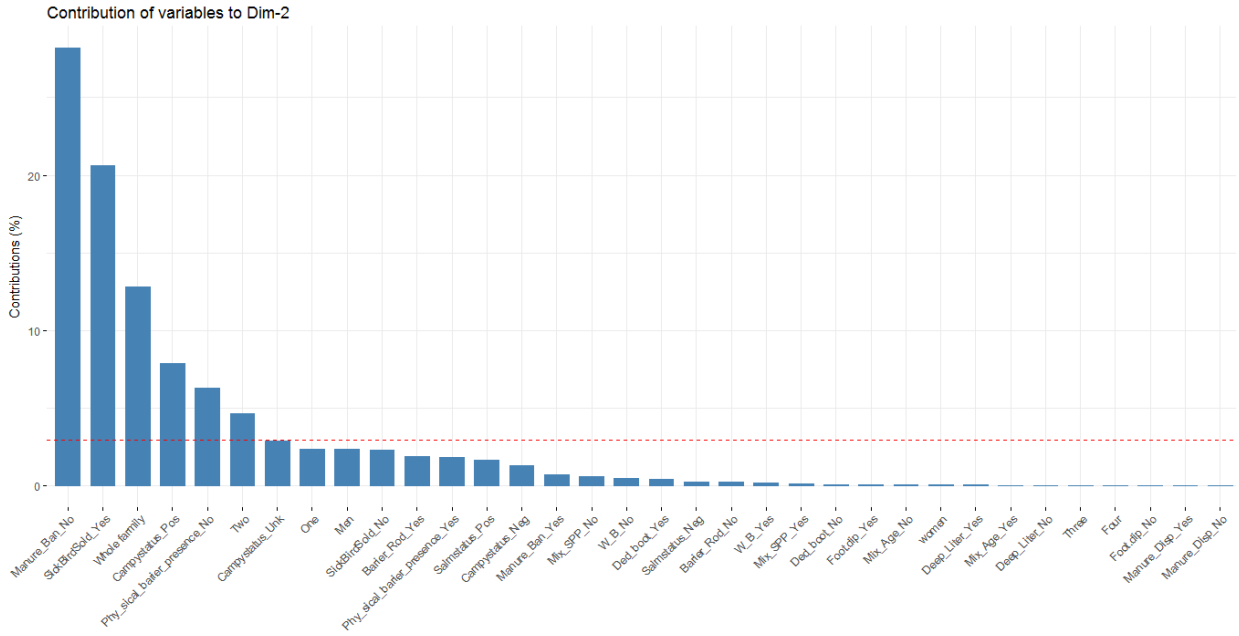


Figure A1 6: contributing to Dimension 2, bars above red dotted line are contributing most to this dimension

R command

corrplot(var$contrib, is.corr = FALSE)
fviz_contrib(dBIO.mca, choice = "var", axes = 2)

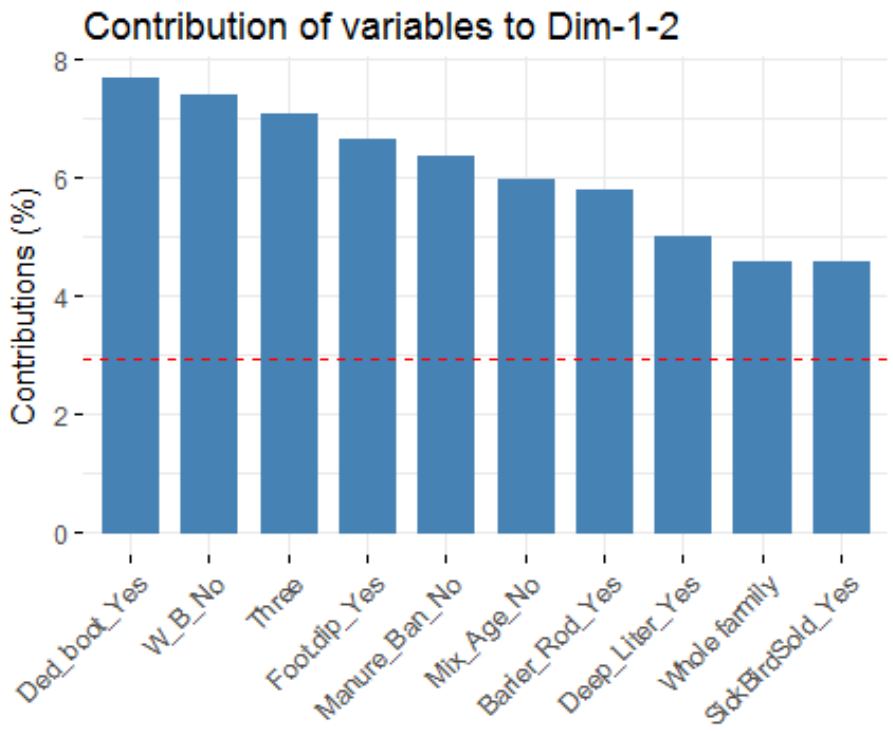


Figure A1 7: 10 variables that contributes most to dimension 1and 2

R-command

fviz_contrib (dBIO.mca, choice = "var", axes = 1:2, top = 10)

dBIO.mca =MCA (dBIO, method = "burt", quali.sup=1:1)

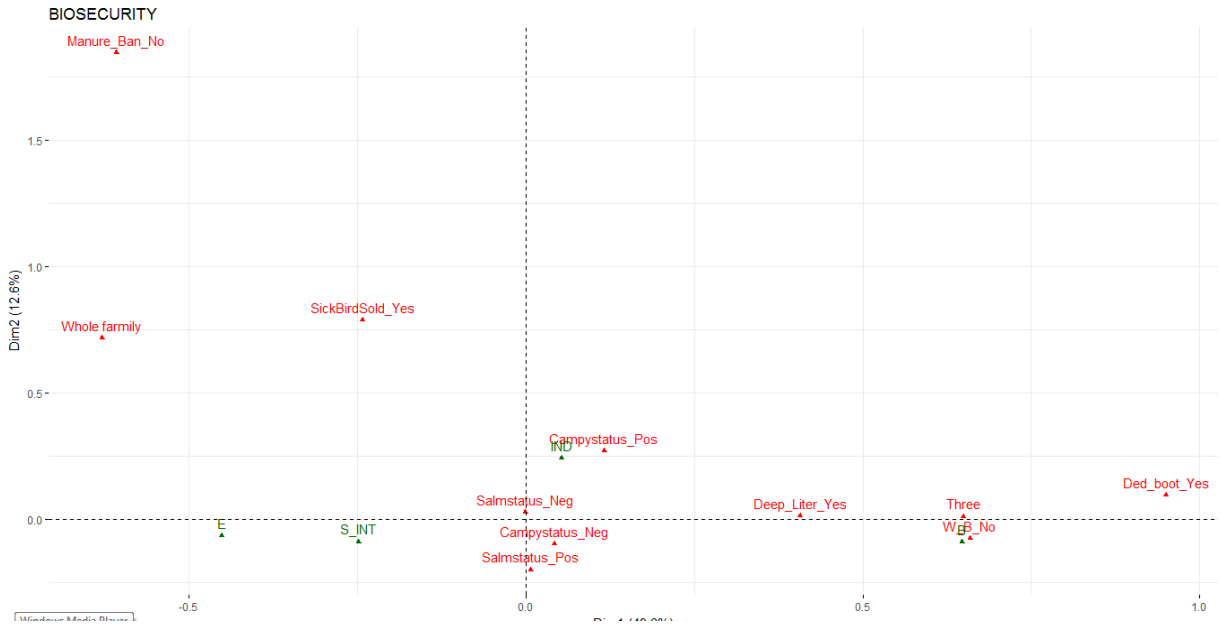


Figure A1: Top contributing categories (Biosecurity) to the variance (*Campylobacter*, *Salmonella* farm status and production system type were included as variable of interest) (NB: "Three" -Three or more chicken houses in one farm, "Salmstatus_Pos"= *Salmonella* positive, "Campystatus_Pos"= *Campylobacter* positive, "Manure ban _No"= Farm does not have dedicated place for manure storage/disposal, "Whole family"=Farm management activity done by any member of the farmily, "SickBirdSold_Yes"=Farmers sale clinically sick chicken, "WB.No"= No wild birds observed in the farm, "Deep_Liter_Yes"= Farms that use deep liter system, "Ded_boot_Yes"= Farms that use dedicated boots, "B"=Broiller farm, "E"=Extensive farm, S_INT"= Semi intensive farm, "IND"= Intensive Indiginous farm)

C) MCA FOR VARIABLES RELATED TO EXTENSION SERVICE DELIVARY

Only variables concerning mainly with extension service provision in the area, farmers source of information, source of finance for training and quality of extension service has been included 10 variables that contribute most to dimension 1and 2 were included to get the final MCA graph.

Table A 4: Extension delivery related variables extracted from table 4

NO	VARIABLES	CODE IN MCA	NUMBER OF LEVELS
1	Farmers receiving extension services	"Ext_Services"	2
	Main extension service provider		
2	Government	"EG"	2
3	Non -governmental organization	"E_NG"	2
4	Private practitioners	"E_P"	2
	Information Sources		
5	Input suppliers	"Infor_sourse_input"	2
6	Social media	"Infor_source_socia"	2
7	Farmers field schools	"Infor_source_FFS	2
8	Colleagues	"Infor_Source_collegue"	2
9	Who finance training	"Who_finance"	4
10	Quality of extension service	"Rank_Ex_Services"	2
11	Farm positive to *Salmonella*	"*Salmonella* Status"	2
12	Farm positive to *Campylobacter*	"Campy status"	2
13	Production system	"Prod_system"	4
14	Traing type	"Training_Type"	2
15	Member group	"Member_Grp"	2
16	Kind of group	"Kind_Grp"	2
17	Skills in poultry	"SkillsPoultry"	2

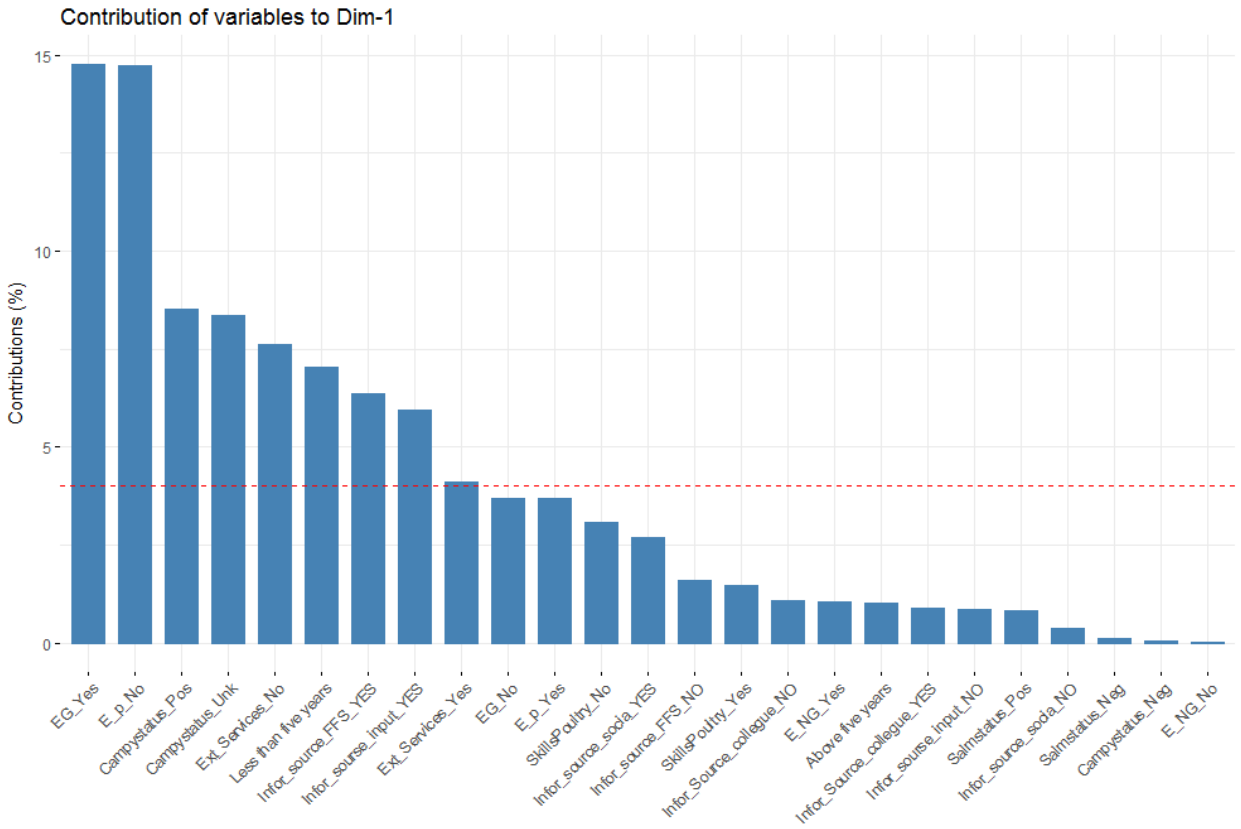


Figure A1 8: Variables contributing to Dimension 1, bars above red dotted line are contributing most to this dimension.

R command: fviz_contrib (dExTT.mca, choice = "var", axes = 1)

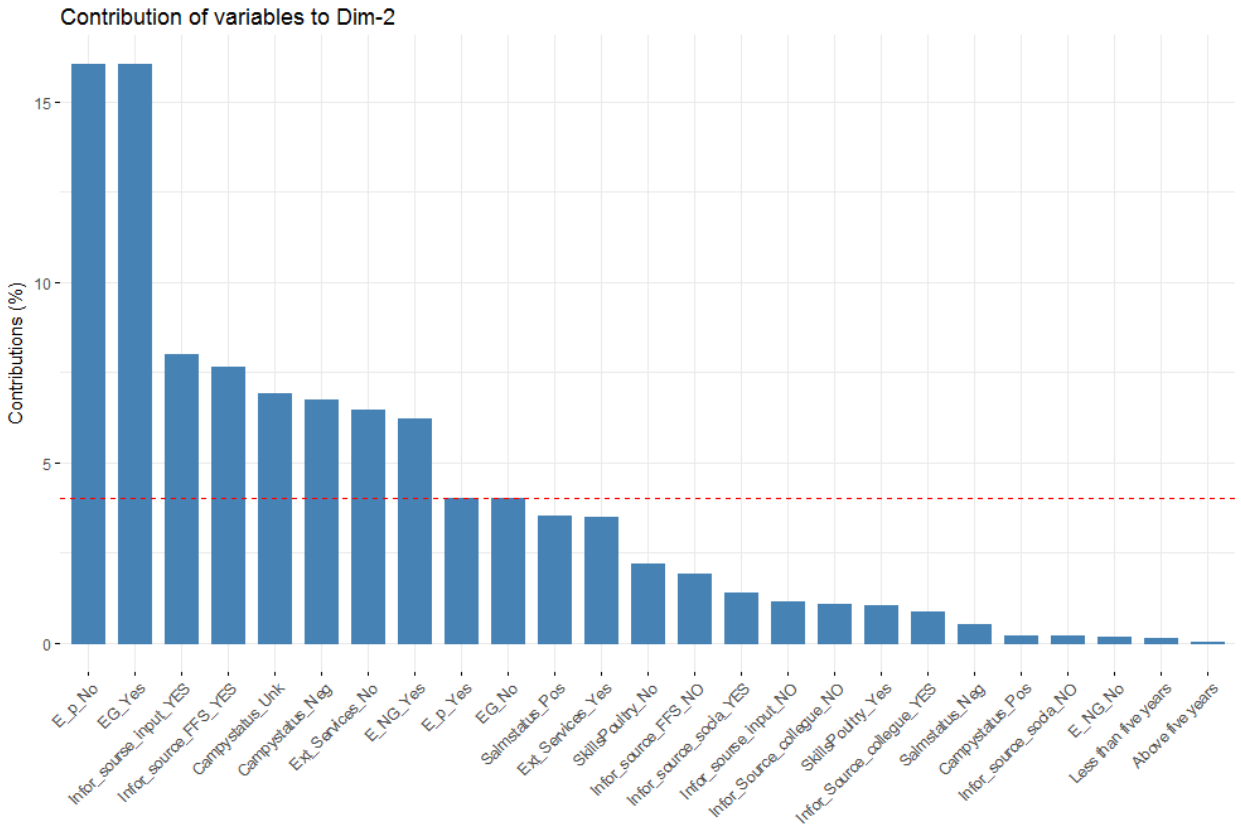


Figure A1 9: Variables contributing to Dimension 2, bars above red dotted line are contributing most to this dimension.

R- Command: fviz_contrib (dExTT.mca, choice = "var", axes = 1)

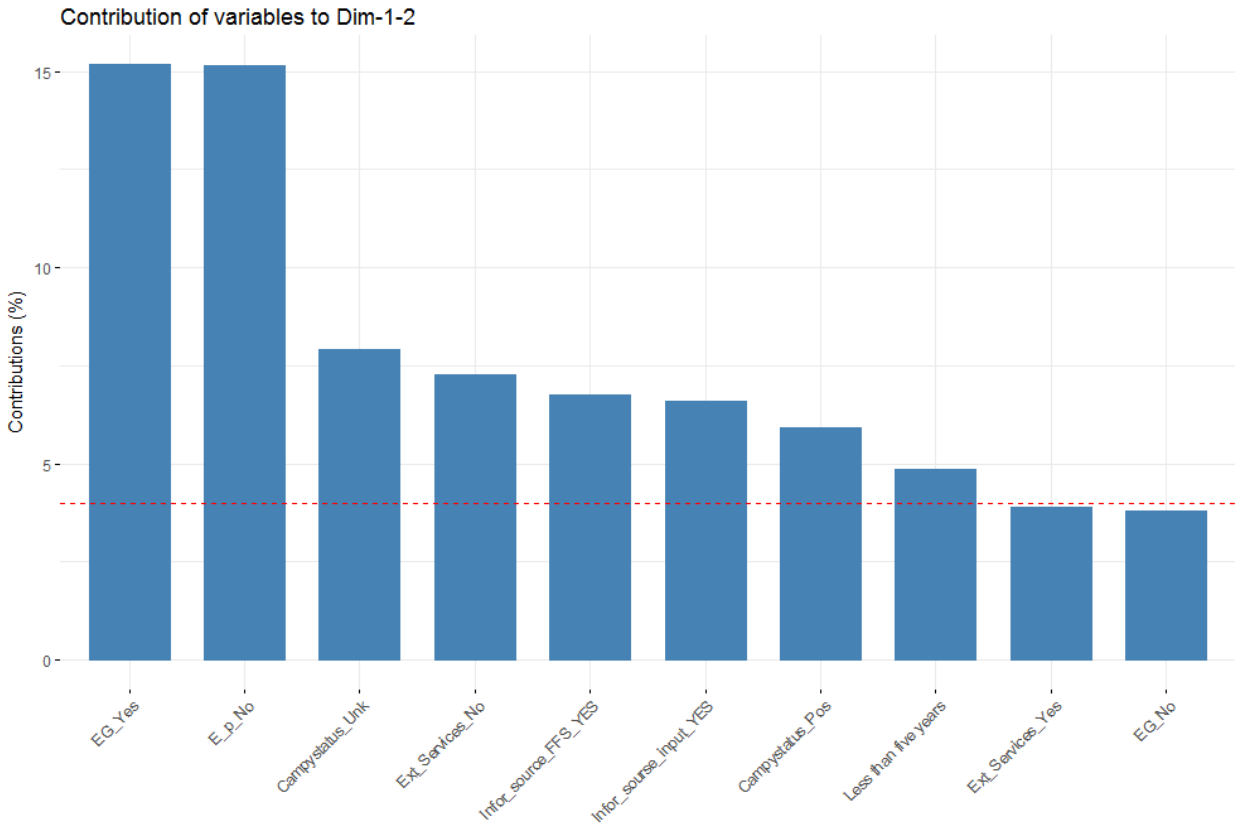


Figure A1 10: 10 variables that contributes most to dimension 1and 2

R command: fviz_contrib (dExTT.mca, choice = "var", axes = 1:2, top = 10)

datex4.mca <- MCA (datex4, method = "burt", quali.sup=1:1)

Table A 5: Eigen values for extension delivery related variables variables as displayed in each dimension.

Dimension	eigenvalue	Variance. Percent	cumulative.variance.percent
Dim.1	0.0478	35.8801	35.8801
Dim.2	0.0219	16.4153	52.2955
Dim.3	0.0171	12.8550	65.1505
Dim.4	0.0158	11.8284	76.9789
Dim.5	0.0095	7.0938	84.0727
Dim.6	0.0064	4.8014	88.8741

R-command: eigenvalues <- get_eigenvalue (datex4.mca) head (round (eigenvalues, 4))

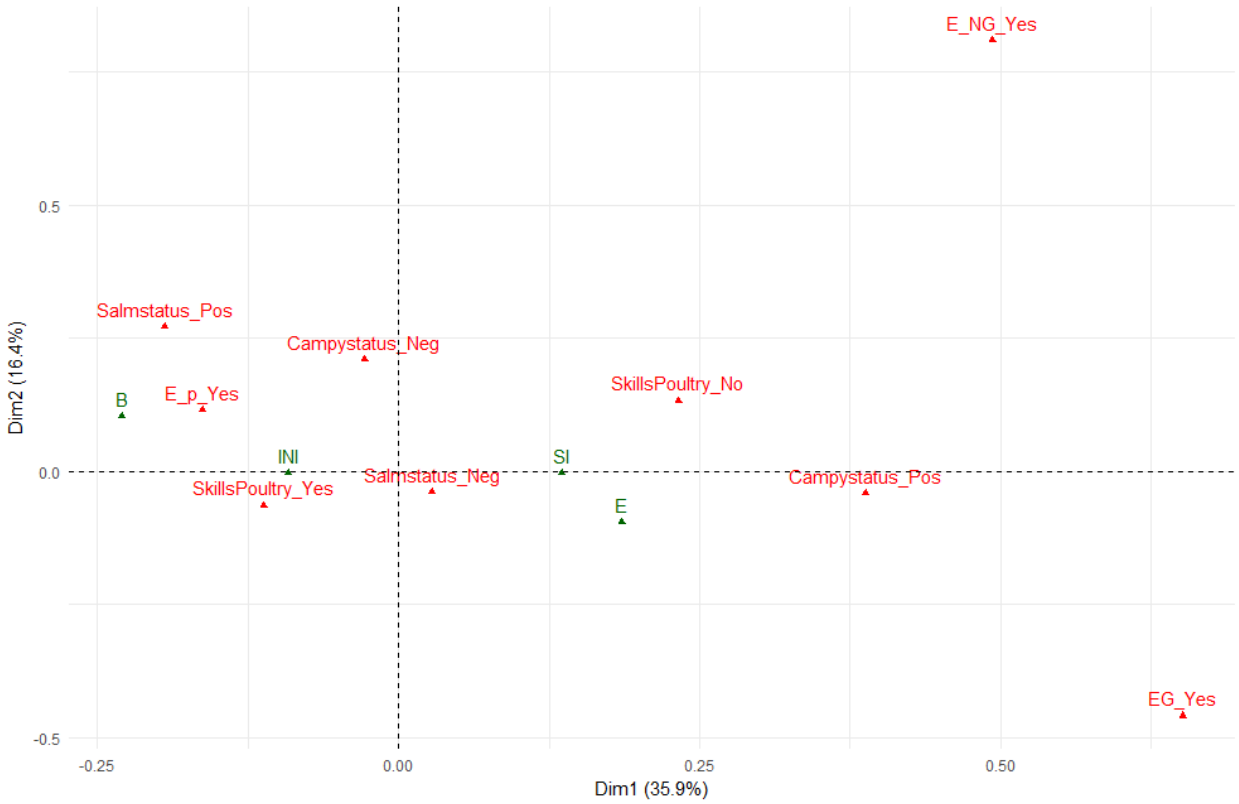


Figure A1 1: MCA graph for extension related delivery variables reduced for better viewing (NB:"EG_Yes"= main extension service provider is Government, "SkilsPoultry_Yes" = Farmers with skills in poultry production "E_NG_Yes"=Source of information was farmers field school gathering, "E_P_Yes" =main extension service provider was private sector,"B"=Broiller, "IN"=Intensive Indiginous,"SI"=Semi Intensive, "E"= Extensive).

Appendix 6: Research output

a) Feedback report

The research project was sponsored by the HAZEL project in collaboration with University of Glasgow and Nelson Mandela African Institution of Science and Technology in Arusha. This activity was planned for 40 house hold poultry keepers and 10 Ward Extension officers from the wards in which the research was conducted. The main objective of the feedback was to create awareness among poultry keepers and collect their views. Participatory approaches were used, which include group discussion with the help of leading questions. Farmers were asked to rank the most important problems that face their poultry production by using pair wise ranking as a tool. Farmers and extension officers were allowed to present on the second day. The purpose of the leading questions provided by facilitator (Mr. Emmanuel Sindiyo) was to identify more burning issues in poultry production. The feedback took 2 days for farmers and 2 days for Extension officers. Training Venue was at Engutoto for farmers and at Veterinary Investigation Centre (VIC) for Ward Extension officers. All participants were consented to photos being taken knowing that the photos might be used for reports or publications

Sessions.

Day 1

-Opening session
-Self introduction
-Overview of the feedback information
-Overview of poultry production in peri- urban settings
-Results presentation in brief
-Group discussion

Day 2

-Group presentation
-Discussion
-Wrapping up

Feedback session

Overview of the research project, presented by Mr. Emmanuel Sindiyo (Msc Student). He presented on the objective of the training, which was to create awareness of poultry management practices under 4 production systems (Broiler, intensive indigenous, Semi-intensive, Extensive). Issues of biosecurity and extension service delivery were discussed including the contribution of poultry to social economic aspect of the farmers' life.

Advantages of poultry keeping

The following was the outline for the presentation

- Poultry as a source of protein
- Poultry as a source of finance
- Social economic importance of poultry
- Diseases of economic importance (Newcastle, Gumborro, pox, coccidiocis)
- Zoonosis (*Campylobacter* and *Salmonella*)
- Urbanization with poultry production
- Extension services provision

Reactions/Questions

One of the participants from Sombetini Ward indicated that there are a lot of problems that face poultry production in Arusha and Tanzania as a whole. He continues to narrate that there is an emerging good number of local hatcheries which are under poor management, they do not have specific parent stock, and they do collect eggs from different parts of Arusha even outside Arusha district.

Extension officer from Themi ward complained about the existence of unknown paraprofessionals in different corners of Arusha, including input suppliers, who also provide livestock services for the purpose of selling their inputs.

Question by facilitator to Extension officers

Base on my results, as covered in the presentation, we observed that poultry keepers especially those who are involved in intensive production they do not get extension services from Government relative to private sector. Why?

Answers from Extension officers

Different answers were given

- We are not available on time to the farmer so farmers look for alternative
- We are not known by them since we were not introduced to them by ward executive officers
- When we arrive to the farm we just give them advice, we cannot do treatment
- We do not have transport, extension kits, drugs and medicine
- We are not mentored
- Chain of command is contradictory so it is difficult to implement our action plans, many times extension officers are involved in other activities as directed by Ward extension officers
- Availability of good number of private practitioners including input suppliers near to farmers

Feedback to farmers

The presentation was not different from that given to the extension officers, what we have changed were leading questions for discussion.

Farmers were asked to identify and rank the major problems that are facing their poultry production and productivity, and if solved they will be happy

1. Reliable source of drugs
2. Reliable source of feed and feed resources for mixing chicken feed
3. Reliable source of daily old chicks
4. Good extension services

Question

Farmers were asked to give reasons why the majority of them do not use Government extension services

The following are reactions from farmers

- When we call them they do give a lot of excuses-they do not come on time
- If they come they just give us advice with no treatment

One farmer from Engutoto narrated that *"when an animal is sick it takes even 2 days for an extension officer to come to my home place. But I have good example: in 1980s*

to 1990s extension officers were very active and when you called them, they used to come with extension kit full of everything but now days is different"

Another farmer from Lemara Ward said that *"Despite the fact that we are getting service from private/input suppliers the quality is not good enough. Sometimes they used to prescribe any drug provided that it is available in his/her shop"*

Way forward

Extension officers

- Farmers' field school on livestock management should be emphasized like in crop sub sector
- Extension officers should be motivated in terms of transport, extension kits
- It is better to change the line of command to reduce bureaucracy
- Inspection of animal inputs should be emphasized
- Feedback from research findings in our area are important

Farmers

- We need to have more information/advice from right source so that we can push on our business
- We need to be assured with animal inputs
- We ask the government to inspect present local hatcheries but also parent stocks
- Market for our products

Figure A 1: Extension Officers in group discussion at VIC-Arusha

Figure A 2: Extension Officers in group discussion at VIC-Arusha

Figure A 3: Extension Officer presenting key issues for discussion

Figure A 4: Farmers discussing major problem that face their poultry production

Figure A 5: Presentation by farmers

Figure A 6: Extension officer giving vote of thanks.

She said “It is my first time in my 25yrs working experience to receive a feedback from researchers so we thank you very much and send our message to your sponsors and the university, we welcome you again”

b) Poster

[Presented to 34th Tanzania veterinary association (TVA) Scientific Conference held in Arusha International Conference Centre (AICC) (see Abstract) from 6th to 8th December 2016]

Risk factors associated with prevalence of *Salmonella* and *Campylobacter* in chicken from different production systems in Arusha district

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Despite their important role in the economy and social life in Tanzania, poultry also exposes the person, environment and consumers to agents of zoonotic infections and food-borne diseases. Non-typhoidal *Salmonella* and *Campylobacter* spp. are two of the most important food-borne zoonotic pathogens. Risk factors at the farm level that are associated with the occurrence of *Salmonella* spp. and *Campylobacter* spp. in chicken from different rearing system in Tanzania are not well documented. This study is designed to assess risk factors associated with prevalence of *Salmonella* and *Campylobacter* in chickens from different production systems in Arusha District. The study will involve collection of environmental samples and cloacal swabs for laboratory investigation. Collection of information on management practices in each production system will involve a cross-sectional survey and in-depth interviewing of poultry keepers using semi-structured questionnaires. The information will help in advising poultry farmers on better management practices to reduce the *Campylobacter* and *Salmonella* load in chicken and along the food chain. Ten of 25 wards will be randomly selected. In each ward, 1 household will be selected per production system (Intensive Broiler, Intensive Indigenous, Semi-intensive indigenous, and Extensive Indigenous). The total number of farmers to be interviewed is 40 and 400 chickens will be sampled for cloacal swabbing. For environmental samples, one pair of boot swabs will be collected from each farm. The samples will be analysed in the KCRI Zoonoses laboratory using standard bacteriology methods. Surveys will be completed in December and preliminary results will be presented.

Key words: chicken, *Salmonella*, *Campylobacter*, risk factors, questionnaire, survey

(c) Manuscript

ORIGINAL ARTICLE

Food Safety, Health Management, and Biosecurity Characteristics of Poultry Farms in Arusha City, Northern Tanzania, Along a Gradient of Intensification

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ABSTRACT

Background: With the growth, urbanisation, and changing consumption patterns of Tanzania's human population, new livestock production systems are emerging. Intensification of poultry production may result in opportunities and threats for food safety, such as improved awareness of biosecurity or increasing prevalence of foodborne pathogens including non- typhoidal Salmonella or Campylobacter spp. We conducted a semiquantitative analysis of poultry production systems in northern Tanzania, with emphasis on biosecurity, health management practices, and prevalence of foodborne pathogens, to gain insight into potential associations between intensification and food safety.

Methods: Interviews were conducted with managers of 40 poultry farms, with equal representation of 4 production sys- tems (extensive, semi-intensive, or intensive production with indigenous chickens, and broiler farming). Per farm, up to10 birds (total, 386) were tested for cloacal shedding of nontyphoidal Salmonella, with a subset of farms tested for Campylobacter. Data were analysed using univariate statistics, and results were discussed during feedback workshops with participating farmers and extension officers.

Results: Clear differences existed between farm types with regard to implementation of biosecurity and health manage- ment practices and use of extension services. By contrast, prevalence of foodborne pathogens (6 of 40 farms or 15% for nontyphoidal Salmonella and 13 of 26 farms or 50% for Campylobacter spp.) was not farm-type specific, indicating that it is driven by other factors. Across farming systems, knowledge and awareness of the presence of antimicrobials in poul- try feed and the need to abide by post-treatment withdrawal times were limited, as was access to impartial professional advice regarding treatment.

Conclusion: Different control measures may be needed to protect poultry health compared to public health, and improvements in information provision may be needed for both.

INTRODUCTION

Urbanisation in Tanzania increased from 5.7% in 1967 to 29.1% in 2012, and urban areas absorbed 12 million people out of a total growth of 31.6 million over that period.[1] Urbanisation is associated with a growth in mean wealth – the value of assets owned – per capita, which increased from US $250 in 2004 to US $480 in 2012.[2] Urbanisation and wealth drive chicken meat consumption, which is skewed towards medium- to high-income populations in urban areas.[3]

Tanzania has an estimated population of more than 43 million chickens.[4] Considering demographic developments in the human population, an increase in poultry production can be anticipated. Indeed, in urban areas, such as Arusha Urban District, traditional extensive backyard poultry farming for home consumption is increasingly complemented by semi-intensive and intensive farming systems, with sales of poultry meat to individual customers, retailers, and caterers. While the majority of chickens – estimated at 96% of the population – belong to indigenous breeds, intensive

were completed before sample collection for 2 reasons: to explain in advance to the farmer how the sampling would take place and to allow for sampling of all farms within a ward (1 each for extensive, semi-intensive, intensive, and broiler production systems) in a single day. The latter was deemed important to avoid temporal bias in culture results from different production systems and would not have been possible if questionnaires also had to be conducted on the same day. Geographical positioning system data were collected for each household using an eTrex 10 device (Garmin, Southampton, UK). Sampling of chickens and their environment was conducted once a week to allow sufficient time for sample processing between sample collection days. Chickens were handled gently to avoid injury, in compliance with the United Republic of Tanzania's Animal Welfare Act no. 19, part V, section 40-48, 2008.[19] Cloacal swabs were collected by inserting the entire tip of a swab into the cloaca of a chicken and applying gentle pressure against the mucosal surface while swabbing in a circular motion. Each chicken was swabbed twice, once with a plain Amies swab and once with an Amies charcoal swab (Thermo Fisher Scientific Newport, UK). Swabs were removed gently and immediately inserted into the respective Amies tubes, and then labelled and stored in cool boxes with ice packs before being transported to the laboratory for analysis within 5 hours – the time between the first sample collection and arrival at the laboratory. Environmental samples were collected by using 1 pair of boot cover swabs (BTSW-001 DRY Sterile Boot Cover Swab for Sampling Poultry Housing, Solar Biologicals Inc., Newark, NJ, USA) per farm and walking along the diagonals of the chicken house or yard. Dry boot cover swabs were used rather than premoistened swabs to avoid bacterial growth prior to use, which was deemed a risk under Tanzanian temperature and moisture conditions. Boot socks were worn over boot covers (Fearing Disposable Boot Covers, Smiths Animal Health, Ashbourne, UK) as per the directions of the boot sock manufacturer. Used boot cover swabs were stored in stomacher bags and transported to the laboratory together with the swabs. After collection of environmental samples and cloacal swabs on a farm and before visiting the next farm, all disposable personal protective equipment was changed, and hands and boots were disinfected using 70% ethanol.

FIGURE 1. Examples of Poultry Production Systems at Different Levels of Intensification

(A) Extensive indigenous poultry production; (B) semi-intensive indigenous poultry production; (C) intensive indigenous poultry production; (D) broiler production

Photos: E. Sindiyo (A) and R. Maganga (B, C, D)

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Sample Processing

Samples were processed in the bespoke Zoonoses Unit of the Biotechnology Laboratory at the Kilimanjaro Clinical Research Institute in Moshi, Tanzania.[20] Culture methods were based on recommendations from the Food and Drug Administration's Bacteriological Analytical Manual for Campylobacter and Salmonella.[21,22] The Campylobacter culture was initiated on the day of sample collection. All reagents were obtained from Oxoid (Basingstoke, UK) unless stated otherwise. Amies charcoal swabs were removed from transport containers and tips removed aseptically by cutting them off into a plastic universal tube containing 20 ml Bolton broth supplemented with 5% horse blood (TCS Biosciences, Botoph Claydon, Buckingham, UK) and selective supplement SR0208E, vortexed aseptically for 10 seconds and placed into a micro-aerophilic jar with CampyGen sachets. Samples were incubated at 37G2°C for at least 4 hours before being moved to 42G2°C for a further 42 to 46 hours, and then plated onto modified charcoal cefoperazone deoxycholate agar plates and incubated at 42G2°C in a microaerophilic jar with CampyGen sachets for 48 hours. Plates were examined for typical Campylobacter colonies. Suspect colonies were subcultured onto Columbia blood agar, incubated microaerophilically at 42G2°C for 48 hours, and subjected to oxidase and catalase testing and Gram staining for confirmation.

Samples for Salmonella detection were stored overnight in a refrigerator between 2°C and 8°C. Tips were aseptically removed from the plain Amies swabs the next day, placed in 20 ml buffered peptone water, vortexed for 10 seconds, and incubated at 37G2°C for 18 to 20 hours. A small volume (0.1 ml) of the enriched buffered peptone water was then transferred into 10 ml of Rappaport-Vassiliadis soya peptone broth and incubated at 42G2°C for 24 hours. One loopful (10 1l) of overnight culture was transferred onto xylose lysine deoxycholate agar with 5 1g/ml novobiocin (Sigma-Aldrich, St. Louis, MO, USA) and streaked for isolation. At least 2 typical Salmonella colonies per plate were streaked onto MacConkey agar and incubated overnight at 37G2°C. Lactose-fermenting colonies (those with a pink appearance) were discarded, and nonlactose-fermenting colonies were individually transferred into 5 ml of tryptone broth and incubated at 37G2°C for 4 to 24 hours. Growth from the broth was inoculated onto MacConkey agar to check for purity, then stabbed into lysine iron agar slopes and triple-sugar iron slopes and incubated overnight at 37G2°C to assess phenotype. Kovacks' indole reagent (Merck KGaA, Darmstadt, Germany) was added to the incubated tryptone broth to test for indole production. Presumptive identification of Salmonella isolates was based on negative indole test results, alkaline slant and butt (purple colour) in lysine iron agar, and red slope with yellow butt and gas production on triple-sugar iron slopes. Identity was confirmed by testing with poly-H and poly-O agglutination tests (Statens Serum Institut, Copenhagen, Denmark) and Microbact 12A test strips, following the manufacturers' instructions.

Data Analysis

Data were stored and checked for missing values and outliers in Microsoft Excel (Microsoft, Seattle, WA, USA), with additional processing using Excel for visual analysis and Statistix 10 (Analytical Software, La Jolla, CA, USA) for quantitative analysis. To test for an association between farm type and categorical variables (eg, biosecurity characteristics or health management), chi-square (X^2) analysis was used. Unless stated otherwise, there were 3 degrees of freedom for X^2 testing, based on analysis of binary variables across 4 farm types. Nonparametric Kruskal–Wallis ANOVA was used for continuous variables. Statistical significance was declared at $P<.05$. To generate a map of the study area showing the production system and culture results for each farm, QGIS software version 2.18.3 (https://qgis.org/en/site/) was used.

Feedback Sessions

Two-day feedback sessions were held with poultry keepers and extension officers in Engutoto Ward and at the Arusha Veterinary Investigation Centre, respectively. The aim of the feedback sessions was to share results from the study, create awareness of biosecurity and health management among poultry keepers and extension officers, and to collect their views on current service provision and needs. After initial introductions and presentation of the results, participatory approaches were used, including group discussions guided by questions and opportunities for participants to present their views. Group discussions were facilitated by the first author, who also arranged the farm visits – with help from the extension officers – and administered the questionnaires to the farmers. The first author was selected for this role because of his knowledge of the subject matter, local conditions, and terminology, as well as the rapport that he had developed with the participants through the project; this facilitated informed and open dialogue.

Ethical Approvals

Ethical approval for this work was provided by the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2028) and the Kilimanjaro Christian Medical Centre (Research Ethical Certificate No. 832), as part of the Zoonoses and Emerging Livestock Systems project. Approval to conduct the interviews of human subjects was granted by the University of Glasgow College of Medical, Veterinary and Life Science's Ethics Committee (200140183), and poultry sampling was approved by the University of Glasgow School of Veterinary Medicine Research Ethics Committee (Ref. 56a/16). A letter of approval was provided by the Municipal Council of Arusha Urban District, where the research took place.

All interviewees provided informed consent before participating in the study. Consent was given verbally in the presence of extension officers rather than in writing to prevent exclusion of participants based on literacy.[20] Details that might disclose the identity of participants in the study are not shown.

RESULTS

Prevalence of Foodborne Pathogens

Visits and interviews were conducted at 40 farms, divided over 4 production systems and 10 wards, with 1 farm per production system per ward. Out of a target number of 400 birds, 386 were swabbed: 99 from broiler flocks (9 farms with 10 birds, 1 farm with 9 birds), 99 from intensive flocks (9 farms with 10 birds, 1 farm with 9 birds), 98 from semi-intensive flocks (8 farms with 10 birds, 2 farms with 9 birds), and 90 from extensive flocks (8 farms with 10 birds, 2 farms with 5 birds). Environmental samples were collected from all farms. Six (15%) of 40 farms and 8 (2.1%) of 386 birds tested positive for Salmonella. Increased farm intensification was associated with nonsignificant increases in the numbers of positive farms and birds (Table 1; X^2=2.3, P=.51 at farm level; X^2=4.6, P=.20 at bird level). Due to logistic issues, samples from 26 farms only were tested for Campylobacter, of which 13 (50%) were positive. Animal-level prevalence of Campylobacter (23 of 255 birds or 9.0%) was higher than for Salmonella but without an obvious association with farm intensity (Table 1). Joint occurrence of Salmonella and Campylobacter was detected on 3 farms, as would be expected by chance under the assumption of independence of occurrence of the 2 bacterial genera. The distribution of farms in the study region, including farm type and farm status, with regards to Salmonella and Campylobacter, is shown in Figure 2.

Farmer Demographics

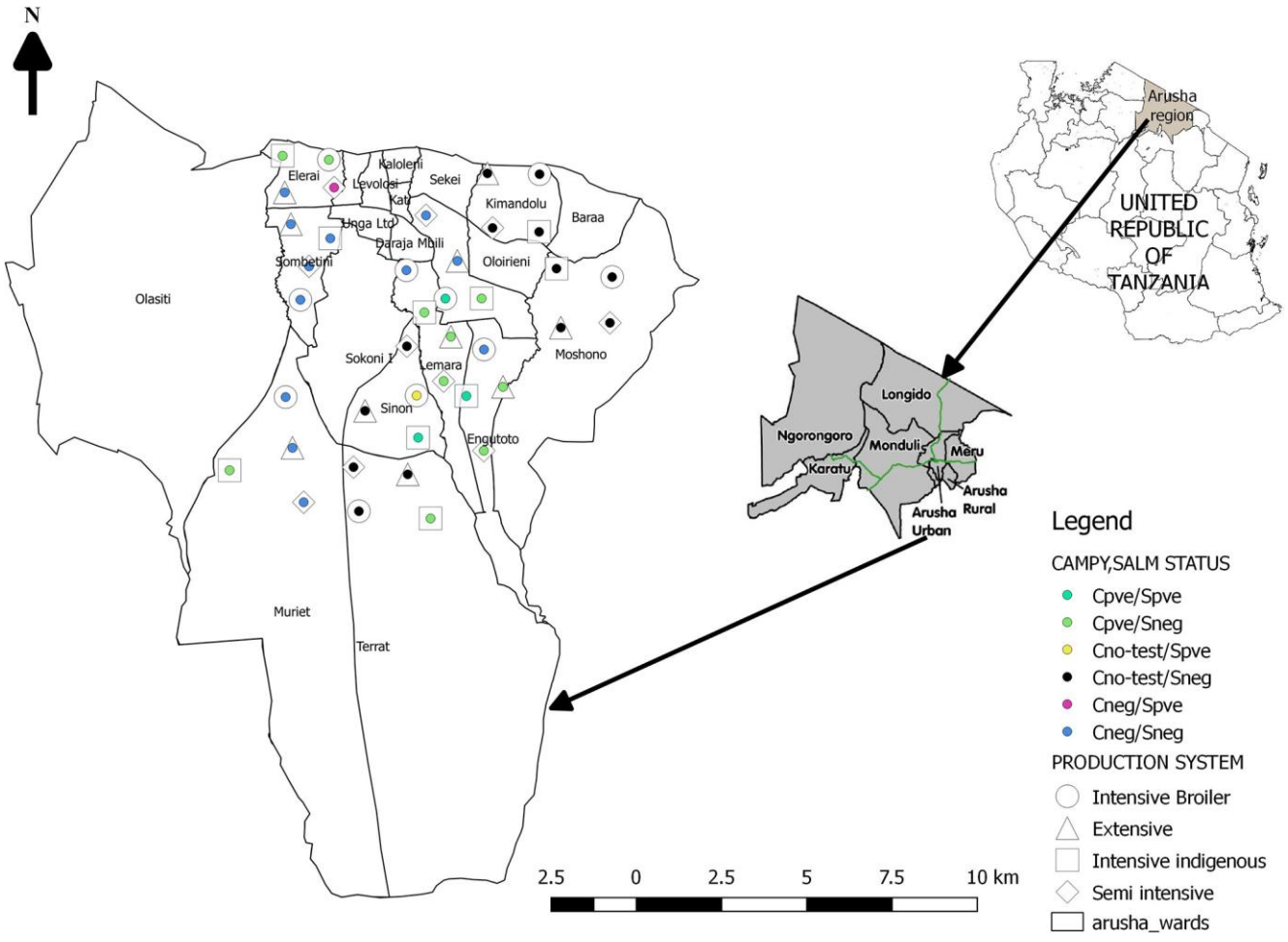
Poultry management was generally the responsibility of women, with a mean of 7 of 10 farms per production system managed by a woman (range, 6 to 8). Only 2 interviewees identified chicken production as their main occupation. Other sources of income included crop production, formal or informal business, and civil service. The majority (n=32, 80.0%) of people responsible for chicken management were over 40 years of age. Of those under 40 years of age, half managed semi-intensive farms, and only 1 was younger than 30. A wide range of education levels was reported, from primary school education (standard 7, equivalent to 7 years of primary education up to age 13), via ordinary and advanced secondary education (form 4 and form 6, respectively), to postsecondary and adult education. Broiler farming was the only sector where none of the respondents reported university-level education, although differences between sectors were not significant. Half of the participants

TABLE 1. Prevalence of Campylobacter spp. and Nontyphoidal Salmonella in Tanzanian Poultry Farms Across a Gradient of Intensification

Pathogen	Farm Type	Farm Level Positive/Tested (%)[a]	Bird Level Positive/Tested (%)	Boot Socks Positive/Tested (%)
Campylobacter spp.	Extensive	2/6	5/55 (9.1)	NA
	Semi-intensive	3/6	7/60 (6.7)	NA
	Intensive	7/8	12/80 (15.0)	NA
	Broiler	1/6	3/60 (5.0)	NA
	All	13/26 (50)	27/255 (10.6)	NA
Nontyphoidal Salmonella	Extensive	0/10	0/90 (0.0)	0/10
	Semi-intensive	1/10	1/98 (1.0)	1/10
	Intensive	2/10	3/99 (3.0)	2/10
	Broiler	2/10	4/99 (4.0)	2/10
	All	5/40 (12.5)[b]	8/386 (2.1)	5/40 (12.5)[b]

[a] Percentage only calculated for denominator values greater than 25.
[b] In total, 6 of 40 farms were positive for Salmonella: 1 semi-intensive farm, 2 intensive farms, and 3 broiler farms (1 farm demonstrated positivity via cloacal swabs only, 1 farm via boot socks only, and 4 farms via both).
Abbreviations: NA, not applicable; spp., several species.

FIGURE 2. Map of Study Area Showing Campylobacter and Salmonella Status by Farm Type and Location



The maps show the position of Arusha Region (grey) within the United Republic of Tanzania and the position of the study area, Arusha Urban within Arusha Region. Wards within Arusha Urban District are shown with approximate (anonymised) farm locations. Farm type is indicated by the shape of the symbol, with coloured dots indicating farm status ("pve", "neg", and "no-test" meaning positive, negative, and not tested, respectively) with regards to Campylobacter (C) and Salmonella (S).

on extensive and semi-intensive farms reported to have skills in poultry production, as did the majority of participants on intensive (8 of 10) and broiler (9 of 10) farms.

Husbandry

On extensive farms, 13 to 75 birds (mean, 39; median, 34) were housed in a single chicken house. On semi-intensive farms, 35 to 105 birds (mean, 57; median, 49) were housed in 1 to 4 houses (median, 1). On intensive farms, 15 to 700 birds (mean, 1999; median 113) were distributed over 1 to 4 houses (median, 2). Finally, on broiler farms, there were 200 to 1,500 birds (mean, 715; median, 600) across 1 to 3 houses (median, 3). The number of birds was significantly higher on broiler farms than on extensive or semi-intensive farms, whereas the number of houses per farm was significantly higher on broiler and intensive farms than on extensive farms (Kruskal–Wallis one-way ANOVA with post-hoc Dunn's pairwise comparison, P<.001 for both analyses). Bedding use reflected intensification of the production system, with litter used on 10, 4, 3, and 1 broiler, intensive, semi-intensive, and extensive farms, respectively (X^2=18.2,

P<.001). Chickens were fed tap water in 5 to 8 farms per farm type, and only extensive farmers used river water. Commercial feed was used on all broiler farms, and home-made feed was used on 9 of 10 intensive farms. Semi-intensive farms used a variety of feed sources, and birds scavenged for food on all extensive farms. All farmers fed their chickens minerals, multivitamins, or both. All producers had purchased their birds, except for 2 extensive producers and 1 semi-intensive producer, who received chickens as gifts.

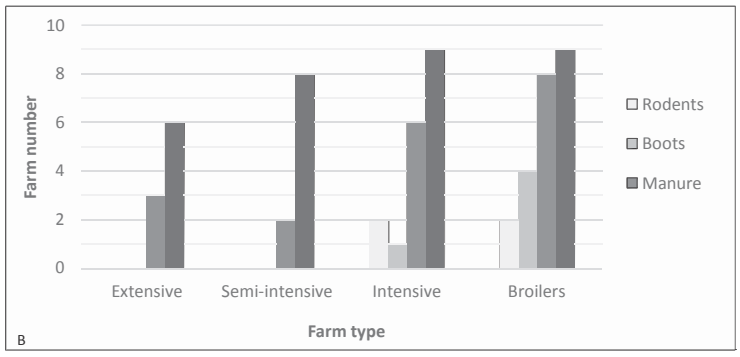
Biosecurity

Biosecurity improved as farm intensification increased (Figure 1 and Figure 3). Mixing of birds of different age groups was common on extensive and semi-intensive farms but not on broiler farms (X^2=14.5, P=.002). With intensification, the number of farms where chickens mixed with other types of fowl decreased (X^2=6.06, P=.11), as did the number of farms where chickens were in contact with ruminant species (cattle, n=14, X^2=21.5, P<.001; goats, n=8, X^2=11.25, P=.01; sheep n=6, X^2=7.1, P=.07). Other types of fowl included ducks, geese and turkeys on n=7, 3 and 3 farms, respectively. Contact with wild birds was common on most farms other than broiler farms (X^2=22.9, P<.001), and all farms reported contact of chickens with rodents, except for a single broiler farm. Contact was also reported with dogs, cats, donkeys, and bats, but not with pigs. The presence of layer hens was reported on half of the extensive farms and most of the semi-intensive and intensive farms but not on broiler farms. All broiler farms practiced the all-in, all-out system, but none of the other farms did. Sick chickens were generally not removed from farms, regardless of farm type, although some were sacrificed (on 2 broiler farms and 1 intensive farm), sold (2 intensive farms), or slaughtered or home consumption (on 1 broiler, 1, intensive, 3 semi-intensive, and 4 extensive farms). Physical barriers limiting access to chickens, separate manure storage, dedicated boots, and rodent barriers were generally more common at the higher levels of

FIGURE 3. Association Between Farm Intensification and Biological and Physical Biosecurity in Poultry Production Systems in Arusha Urban District, Northern Tanzania

Animals	Extensive	Semi-intensiv	Intensive	Broilers
Mixed fow	4	2	1	0
Mixed cattle	9	4	1	0
Mixed age	9	9	6	2
Wild birds	10	10	8	2
Rodents	10	10	10	9
Neighbour	6	10	9	10

Barriers	Extensive	Semi-intensiv	Intensive	Broilers
Footbath	0	0	0	1
Rodents	0	0	2	2
Boots	0	0	1	4
Manure	3	2	6	8
Physical	6	8	9	9



(A) Number of farms with chickens in contact with other animals (other fowl, cattle, chickens of other age groups, or wild birds). (B) Number of farms using biosecurity barriers (ie, rodent barriers, dedicated boots, dedicated manure storage, or physical barriers to access to poultry). Ten farmers were interviewed per farming system.

intensification (Figure 3B). The association with farm type was significant for manure storage (X^2=9.12, P=.028) and use of dedicated boots (X^2=9.83, P=.020), but not for the other barriers, nor for the use of food baths, which was limited to a single broiler farm.

Health Management

Vaccines to prevent viral diseases were commonly used, with half of the farmers using a vaccine against Newcastle disease (Table 2). Vaccination against Newcastle disease was significantly more common on extensive and semi-intensive farms, and pox vaccination was more common on intensive and broiler farms. Half of the farmers reported use of antihelminthics, with a nonsignificant association between antihelminthic administration and farm intensification (Table 2). Antimicrobial use was reported by a clear majority (n=38, 95.0%) of farmers, whereas traditional herbs were predominantly used by extensive farmers. Routine use of antimicrobials was significantly more common on broiler farms than other farm types (P=.002), where antimicrobials were reported to be used occasionally or only when birds were sick. With a few exceptions (1 each among extensive and semi-intensive farms, and 2 among intensive farms), treatments were administered to the entire flock rather than to individual sick birds. The choice of drugs was mostly based on advice from drug sellers, with a minority of farmers primarily relying on veterinary advice or personal experience. Only 1 semi-intensive farmer reported consulting an

TABLE 2. Use and Knowledge of Vaccines and Drugs on Tanzanian Poultry Farms Across a Gradient of Intensification

	Farm Type				Statistics	
Topic	Total n	Broiler n	Intensive n	Semi-intensive n	Extensive n	Chi-square	P Value
Vaccines							
Gumboro	1	0	1	0	0	3.1	.38
Newcastle disease	20	0	5	7	8	15.2	.002
Pox	13	6	5	2	0	10.4	unavailable
Drugs							
Antihelminthics	20	7	6	4	3	4.0	.26
Antimicrobials							
Routinely	10	7	1	1	1	14.4	.002
Occasionally	12	2	5	4	1	4.8	.19
When birds are sick	16	1	4	5	6	5.8	.12
Traditional herbs	8	1	2	0	5	8.8	.03
Drug choice based on							
Personal experience	9	4	2	3	0	5.0	.17
Drug seller's advice	21	4	4	5	8	4.3	.23
Veterinary advice	9	2	4	2	1	2.5	.48
Knowledge of							
Antimicrobials in poultry feed	4	2	1	1	0	2.2	.53
Residue impact on people	17	5	4	4	4	0.3	.99
Withdrawal time							
Aware	17	7	5	3	3	4.4	.22
Abides	9	3	3	1	2	1.6	.66

extension officer before treatment. Few farmers were aware that poultry feed might contain antimicrobials. Across farming systems, almost half of all farmers said that they were aware of the impact of antimicrobial residue on human health and the existence of withdrawal times after antimicrobial use, but only a quarter abided by rules around withdrawal times (Table 2).

Extension and Training

All farmers expressed the need to receive information on poultry keeping. Most farmers were members of 1 or 2 professional groups, including farmer field schools or poultry associations, but only a minority considered this useful. Most farmers – particularly broiler farmers –relied on input suppliers for extension services. Some farmers – particularly those on extensive farms – relied on government extension officers for extension services. Information on poultry farming was mostly obtained from colleagues and occasionally from farmer field schools, input suppliers, or social media. None of the associations between information source and farm type were significant (Table 3). Farmers' own resources were the most common sources of funding for training on all but extensive farms, where the government was the most common source of funding for access to information (data not shown). Nongovernmental organisations occasionally funded access to information, but they were never cited as the main source of information.

Issues impacting the use of extension services by farmers were identified by farmers and extension officers in separate feedback sessions. The 2 major issues identified by both groups were timeliness of the extension officers' responses to requests from farmers and the fact that extension officers provide advice without being able to offer treatment or vaccination. Timeliness of service provision was affected by a

TABLE 3. Engagement of Poultry Farmers With Farmers Groups, Extension and Information Providers, and Vaccine Suppliers Across a Gradient of Farm Intensification in Northern Tanzania

	Farm Type				Statistics[a]	
Topic	Total n	Broiler n	Intensive n	Semi-intensive n	Extensive n	Chi-square	P Value
Farm group membership[b]	32	6	10	8	8	5.0	.17
Farmer field school	25	8	5	6	6	2.0	.57
Poultry association	15	2	5	4	4	1.3	.72
Useful	10	3	2	1	4	2.7	.46
Main extension provider							
Government	8	0	1	3	4	6.3	.10
Input supplier	21	8	5	4	4	4.3	.23
Nongovernmental organisation	1	0	1	0	0	3.1	.38
Information sources							
Farmer field school	8	1	1	2	1	0.7	.88
Input supplier	5	1	1	2	1	0.7	.88
Social media	5	1	1	3	3	2.5	.48
Colleagues	22	7	7	3	5	4.4	.22
Vaccine provider							
Government extension	1	0	0	1	0	3.1	.38
Input supplier	38	10	10	9	9	2.1	.55

[a] P values indicate significance of an association between farm type and engagement (yes/no) based on chi-square analysis.
[b] Ten farmers were interviewed per farm type, and numbers indicate the farmers using the specified membership or service. Some farmers did not use any of the service providers listed, so numbers may not add up to 10 per farm type.

lack of available transport and by competing demands on the extension officers' time, while the quality of the service that could be offered was affected by a lack of mentoring, extension kits, and medicines. An additional issue was the lack of appropriate introductions of extension officers to farmers by the relevant authorities. Private veterinarians and input suppliers can provide advice more quickly. Moreover, they have the ability to offer treatment products, although farmers recognised that they sometimes prescribe drugs that are available in their shop without due consideration of the suitability of the treatment. Suggestions for improvement largely revolved around related issues, including provision of transport and extension kits and changes to the chain of command for extension officers. In addition to health information, farmers desired information that could help them develop their business and access markets, as well as government involvement in inspections of hatcheries and parent stock. Feedback from research was particularly valued by extension officers and was summarised as follows in a vote of thanks on behalf of the group: "It is my first time in 25 years working experience to receive feedback from researchers, so we thank you very much and send our message to your sponsors and universities: We welcome you again".

DISCUSSION

Nontyphoidal Salmonella and Campylobacter are important human pathogens in sub-Saharan Africa and may be transmitted through food of animal origin, including poultry products derived from healthy birds. Many foodborne pathogens of people are commensals in the gastrointestinal tracts of animals, ie, bacteria that are carried without causing disease. Indeed, all Salmonella and Campylobacter isolates in the current study originated from clinically healthy birds. Small-scale outbreaks of foodborne disease due to contamination of human food with enteric commensals from animals have probably occurred throughout human history. They gained prominence in public health and scientific research in the latter part of the 20th century, when large outbreaks of salmonellosis and listeriosis in the United States and mortality due to Escherichia coli O157 stimulated public awareness and development of the scientific discipline of food safety.[23–25] Several major foodborne disease outbreaks in the United States and the UK occurred as a result of intensification and expansion of food production and distribution networks – processes that are currently taking place in much of sub-Saharan Africa.[24,25]

Traditional poultry keeping practices in Tanzania are changing as the country's poultry industry expands to meet the demands of a growing and increasingly urban consumer population. While intensification of food production is needed to provide food security, it must not come at the expense of food safety. Development and implementation of hazard analysis critical control point (HACCP) approaches across networks in the food industry may limit the risk of foodborne disease. For example, implementation of the Lion Code to control Salmonella Enteritidis in the British poultry industry has been followed by a significant decrease in human infections with this organism.[26,27] In Africa, intensification of poultry production has been linked with increased prevalence of Salmonella and decreased prevalence of Campylobacter, but little is known about the association between farm management, biosecurity, and pathogen prevalence in relation to the emerging poultry systems in Tanzania.[8,17] Moreover, it is largely unknown how farmers access information on these topics.

Specific Risk Factors for the Presence of Foodborne Pathogens are Difficult to Identify

The prevalence of Salmonella in clinically healthy poultry was low in our study in Arusha, which is a positive outcome. A previous study of Salmonella in Tanzanian poultry also found a low prevalence, but that study focused on Salmonella enterica subspecies enterica serovar Gallinarum in layer hens.[28] In our study, layers were not included, and serotyping of isolates was beyond the scope of this work, making it difficult to compare data across studies. A range of biosecurity measures were considered in our study, and many differed between farm types, including mixing of chickens with wild birds or ruminants. Although livestock, wild birds, and other wildlife may act as a source of Salmonella and introduce the organism into poultry flocks, we observed no association between farm types with different biosecurity levels and Salmonella prevalence.[7,29]

A lack of identifiable risk factors was also reported in a large study from Canada, where permanent locking of the poultry house was the only factor significantly associated with Salmonella prevalence.[16] This risk factor was interpreted as a proxy for general biosecurity measures, but specific measures, such as boot washing, professional rodent control, or absence of contact with other host species were not significant.[16] An alternative source of Salmonella exposure for chickens is poultry feed. A recent study on commercially produced chicken feeds from 3 feed mills in Dar es Salaam, Tanzania, showed that Salmonella prevalence ranged from 15% to 48% of feed bags, with significant differences between feed mills.[30] This suggests that the "farm-to-fork" or "stable-to-table" concept should include poultry feed, as is the case with the British approach to Salmonella control.[26] To determine the importance of feed as a source of Salmonella carriage in chickens or the importance of carriage in chickens as a source of human foodborne disease, strain typing of isolates from feed, chickens, and people would be required. In Burkina Faso, poultry, cattle, and pigs were shown to have similar levels of intestinal carriage of Salmonella, but only poultry isolates were genetically similar to those from humans, implicating poultry as the most likely source of human pathogens.[7]

Flock-level prevalence of Campylobacter carriage was 50% in our study, again without noticeable health impacts on the animals and without identification of specific risk factors, making it difficult to provide reasons and recommendations for Campylobacter control based on poultry health alone. Moreover, occurrence of Salmonella and Campylobacter was independent, suggesting that they are driven by different underlying processes and may require distinct control strategies. The fact that foodborne pathogens do not cause disease in animals poses a significant challenge because interventions that contribute to improved food safety and public health do not necessarily provide benefits to animal health. This is illustrated by the situation with E. coli O157:H7 in the UK, where vaccination of cattle would have major public health benefits but no animal health benefits, and uptake by farmers is low due to lack of economic incentives.[31] Likewise, resource-constrained poultry producers in Tanzania may have low incentive to invest in control of foodborne pathogens that do not affect the health of their birds.

Antimicrobial Use is Common in Poultry Production and May Pose a Risk to Public Health

In addition to the issues of food safety and food security, both of which should be considered One Health issues, a third One Health issue was identified through questionnaires: a lack of guidance and knowledge around the use of antimicrobials. Fewer than half of the farmers were aware of the existence of withdrawal times after antimicrobial use, and even fewer abided by the withdrawal guidelines. Considering global concerns about antimicrobial resistance (AMR), the observed lack of awareness and compliance with withdrawal times needs to be addressed. Awareness and compliance were more common among intensive and broiler farmers, hinting at potential benefits of intensification in terms of farmer education.

At the same time, broiler farmers were more likely to use antihelminthics and to use antimicrobials routinely. Broiler farmers were also more likely than other farmers to rely on input suppliers for extension services and on colleagues or personal experience for information and treatment decisions. Lack of independent, professional advice could contribute to frequent drug administration, which might contribute to higher selection pressure in favour of AMR, suggesting a potential hazard of farm intensification. The numbers in our study are small and associations are mostly nonsignificant, but the lack of unbiased professional input towards health management and treatment decisions may warrant more thorough socio-anthropological investigation of this issue. Tanzania's National Action Plan on AMR includes an analysis of strengths, opportunities, weaknesses, and threats and recognises that inadequate promotion of food safety along the chain and dispensing of antimicrobials by nonprofessionals are recognised as threats to AMR prevention.[32]

Poultry Farmers and Extension Officers Agree on the Need for Improved Service Provision

The importance of communication and access to information and drugs were also raised in feedback workshops. The fact that extension officers offered advice on health management and disease prevention rather than products for disease treatment was seen as a major weakness of the service they provide. This has been a longstanding problem in preventive veterinary medicine throughout the world, and cycles of rise and fall in interest in preventive rather than curative approaches have been described in detail in the UK.[33] Briefly, in times of need, urgency tends to take precedence over long-term consequences, and resources are diverted towards curative approaches. Use of resources for disease prevention is more likely in periods of relative wealth and calm. In Europe, differences still exist between production sectors, whereby preventive health management is now the standard on poultry farms, and cattle practice is often still largely responsive. Currently, only 20% of livestock farmers in Tanzania use livestock services.[34] Policy priorities for improved livestock services were recently identified by means of a livestock field officer survey.[34]

The survey identified better transport, improved balance between administrative and technical duties, and supervision for livestock officers as policy priorities. These priorities were echoed in our feedback workshops. Additional priorities were regulation of fees charged by livestock officers – who may also act as private input suppliers – and better communication between central and local government staff on livestock-related policy.[34] Our data suggest that improvement in communication is not only needed within the government-regulated livestock system but also between the government system and poultry producers, particularly producers in intensive systems. If trends in population growth and urbanisation continue, so will the intensification of poultry production. Considering that the average broiler flock in the study area was almost 20 times as large as the average extensive flock, a growing proportion of poultry meat will originate from broiler farms. Unbiased information on disease prevention and control, along with incentives to limit the use of antimicrobials and the risk of AMR, will become increasingly important as the intensification of poultry production continues.

Limitations

This study had several limitations, such as the limited number of farms per production system and the narrow geographic focus on Arusha Urban District. However, all relevant levels of intensification were represented, and the information obtained from the study has yielded valuable insight into the complexity of managing poultry health and public health in an economically viable manner. Particularly, our results suggest that biosecurity measures, which farmers implement to protect poultry health, are not

directly linked with the prevention of foodborne pathogens, and that different foodborne pathogens may have different drivers of prevalence. A much larger study would be needed to identify risk factors for all relevant poultry health and public health hazards, and it would need to be accompanied by economic studies to understand how to incentivise poultry keepers to take measures to prevent multiple hazards, including those that are not directly related to poultry health. A second limitation of this study is that Salmonella and Campylobacter isolates were not identified to strain level, and they were not compared with isolates of human origin. Thus, their potential contribution to the human disease burden was not demonstrated at the molecular level. Isolates have been archived at Kilimanjaro Clinical Research Institute so that molecular epidemiological investigations can be conducted at a future date.

CONCLUSION

Population growth, urbanisation, and the associated emergence of intensified poultry production systems in Tanzania bring opportunities and risks for poultry farming, public health, and food safety. Based on our findings, biosecurity and awareness of antimicrobial residues is better on large, intensive farms than on small, extensive farms, implying that intensification may bring benefits for poultry health (reduced risk of disease introduction through better biosecurity) and for human health (reduced risk of antimicrobial residue in food for human consumption). In contrast to extensive producers, who receive advice from government extension officers, intensive producers tend to receive poultry health and treatment advice from private commercial suppliers who may have inherent conflicts of interest related to provision of information and products. This could contribute to overuse of antimicrobials and might constitute a risk to public health. Biosecurity measures were not linked to detection of Salmonella or Campylobacter, implying that farm management strategies to protect poultry health do not necessarily protect human health. Separate control strategies may need to be developed to limit the presence of foodborne pathogens. This is further complicated by the fact that occurrence of the 2 pathogens seems to be independent, suggesting that different transmission mechanism and control strategies are involved. For the sake of food security and public health, it seems important that the Tanzanian government develops ways to engage with its emerging poultry production system so that the potential benefits of intensification for biosecurity, food security, and food safety can be reaped without increasing the risk of overuse of antimicrobials.

Acknowledgements: We would like to thank the participating farmers and extension officers for their valuable time and insights, and Nsimbo District Council Director Mwailwa Smith Pangani for support of ES's studies. We are grateful to Ms Dassa Nkini, Ms Mary Ryan, and Mr Fadhili Mshana for administrative and logistic support and to Mr Mike Shand for training in QGIS.

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Peer Reviewed

Competing Interests: None declared. The views expressed in this paper are purely the opinion of the authors based on the study findings and not those of the Ministry of Livestock and Fisheries or other employers.

Funding: This work was funded by the Biotechnology and Biological Sciences Research Council, the Department for International Development, the Economic and Social Research Council, the Medical Research Council, the Natural Environment Research Council, and the Defence Science and Technology Laboratory, under the Zoonoses and Emerging Livestock Systems programme (grant numbers BB/L017679/1 and BB/N503563/1). The funding bodies had no role in designing the study; collecting, analysing, or interpreting the data; and no role in writing the manuscript.

Received: XXXXXX; Accepted: XXXXXX