FOOD SAFETY RISK ASSESSMENT OF THERMOPHILIC *CAMPYLOBACTER* IN BEEF IN ARUSHA MUNICIPALITY, TANZANIA.

BY

EDGAR ANGELUS MAHUNDI

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ABSTRACT

Campylobacter is one of the most important pathogen which cause food borne illnesses in the world. A study on risk assessment of thermophilic *Campylobacter* of beef in Arusha municipality, Tanzania was carried out from January to March 2010. A total of 138 consumers, 35 meat sellers and 25 meat vendors were interviewed on beef purchasing preference, safety during cooking and hygienic practices during consumption. One hundred and sixty swab samples were collected from beef carcasses in butchershops (n=73), roast beef (*Nyamachoma* in Kiswahili) (n=45) and skewer beef (*mishikaki*) selling points (n=42) located in beer bars. All the swab samples were used for isolation of thermophilic *Campylobacter*. The number of customers per hour was recorded in nine *nyamachoma* and nine *mishikaki* centres for seven consecutive days. Data were subjected to a Monte Carlo simulation to determine the likelihood of consuming ready-to-eat beef contaminated with thermophilic *Campylobacter*. There was a contamination rate with thermophilic *Campylobacter* of 24%. The probabilities of consuming contaminated meat with thermophilic *Campylobacter* at *nyamachoma* pubs were 15.5% and at *mishikaki* shops was 34.7%. The total amount of beef sold at nyamachoma pubs in Arusha municipality per day was 3, 595 kg (90% CI: 1, 745-6, 173) and that sold as mishikaki per day was 165 kg (90% CI: 57-328). The exposure rate per person in *nyamachoma* was 0.16% while that in *mishikaki* was 0.017%. Interview result revealed that poor knowledge on campylobacteriosis and lack of training on food hygiene contributes to poor food safety. However, the control measure practiced by food handlers was to wash hands with soap and water in order to prevent food contamination. At homestead food was covered after cooking and consumers ate

food while was still hot. Cross-contamination events were observed in the kitchen whereby knives, utensils, hands of the personnel could probably contribute to contamination with *Campylobacter*. However, public education programmes and consumer awareness on general food hygiene are recommended to reduce potential health risks to the public.

DECLARATION

I, **Edgar Angelus Mahundi**, hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been submitted nor being concurrently submitted for a higher degree award in any other University.

.....Mahundi..... .....2/11/2011.....

Edgar Angelus Mahundi **Date**

(MVM candidate)

The above declaration is confirmed

....7/11/2011.....

Prof. L.R. Kurwijila **Date**

(Supervisor)

....02/11/2011.....

Dr. E.D. Karimuribo **Date**

(Supervisor)

.....ANgowi'..... .....04/11/2011.....

Dr. H. A. Ngowi **Date**

(Supervisor)

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DEDICATION

This work is dedicated to my wife Venchi Gabriel Mwano for her endless love and support.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	-	Percent
°C	-	Degrees *Celsius*
AIDS	-	Acquired Immunodeficiency Syndrome
CAC	-	*Codex Alimentarius* Commission
ELISA	-	Enzyme-Linked Immunosorbent Assay
FAO	-	Food and Agriculture Organization of the United Nations
g	-	gram
GPS	-	Geographical Positioning System
HACCP	-	Hazard Analysis and Critical Control Points
HIV	-	Human Immunodeficiency Virus
Kg	-	Kilogramme
Km	-	Kilometer
m	-	Meter
MAC	-	Ministry of Agriculture and Cooperatives
MLD	-	Ministry of Livestock Development
Mishikaki	-	Skewer beef
Nyamachoma	-	Roast beef
PCR	-	Polymerase Chain Reaction
UMoH	-	Uganda Ministry of Health
URT	-	United Republic of Tanzania
WHO	-	World Health Organization of the United Nations

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Food-borne illnesses are prevalent in most parts of the world, and the cost in terms of human life and suffering is enormous. Contaminated food contributes to 1.5 billion cases of diarrhoea each year, resulting in more than three million premature deaths in the world (WHO, 2005).

The high incidence (14.9%) of diarrhoeal diseases among newborns and young children are indications of poor food hygiene situation in the African region (Adekunle *et al.*, 2009). Although outbreaks of acute food poisoning are frequent in the African region, surveillance is inadequate or nonexistent, which hinders governments' ability to recognize the impact of food contamination on public health (WHO, 2002). While poverty is the underlying cause of consumption of unsafe food in the developing countries, other factors, such as lack of access to clean water, weak government structures, population growth, the rise of Acquired Immunodeficiency Syndrome (AIDS) and other communicable diseases, trade pressure, and poor environmental conditions exacerbate the situation. Lack of national legislation and limited resources to control the quality of foodstuffs further compound the challenges faced by developing countries (Caroline and Nardine, 2005).

Food quality control activities in Tanzania are carried out by departments and agencies located in seven ministries which are the Ministry of Health and Social

Welfare through Tanzania Food and Drugs Authority (TFDA), Ministry of Agriculture and Food Security. Ministry of Livestock Development and Fisheries, Ministry of Natural Resources and Tourism.

Ministry of Regional Administration and Local Governments. Ministry of Industry, Trade and Markets and Ministry of Communication Science and Technology. Tanzania Bureau of Standard is a body that coordinates food standards at national level. This is a Codex contact point which facilitates incorporation of international food safety standards based on Codex standards (WHO. 2005). Tanzania Food and Drugs Authority is a regulatory body which is responsible for controlling quality, safety and effectiveness of food, drugs, cosmetics and medical devices. They have inter-institutional collaboration which involves stakeholders to ensure roles of one institution do not overlap with the other. Unhygienic meat handling may constitute a potential risk of infections to humans. Livestock products may be sources of food-borne zoonoses such as tuberculosis, brucellosis and campylobacteriosis. Thermophilic *Campylobacter* is one of the most frequent isolated bacteria from meat and human beings. Thermophilic *Campylobacter* namely *C.jejuni, C.coli, C.lari, C.upsaliensis* are the major causes of human campylobacteriosis as they account for four million cases of diarrhoeal illnesses worldwide annually (Heymann, 2004).

Most data available on campylobacteriosis in developing countries were collected as a result of support provided by WHO to many laboratories (WHO, 2005). The difficulty in modelling and estimating the risk posed by campylobacteriosis lies on the difference between dose-response of infection and illness. Black (1988)

conducted an experiment using volunteers to model dose-response in the United States of America. In the study, volunteers ingested different dose amounts of milk containing *Campylobacter jejuni* and infection and illness were recorded. Infections in this study showed dependency on dose; however probability of illness decreased with the dose increase.

Using data from two outbreaks of campylobacteriosis due to milk contamination among school children in the Netherlands reported by Van den Brandhof (2003) and in UK as reported by Evans (1996). Teunis (2005) attempted to model the dose-response relationship of illness conditional to infection. However, immunity status seems to affect greatly the dose dependent probability of illness and the model to match all the situations in the world. Many studies in developing countries have shown that children below 5 years acquire immunity and the incidence decrease thereafter, however, the protection is not perfect because cross immunity is less likely to occur (Havelaar, 2009).

Basing on assumption that Tanzania is in East African region and that the population immunity status is similar to that of Uganda therefore, present study used Ugandan data (UMoH, 2006) to model the population immunity status in East Africa.

1.2 Justification

Arusha is estimated to have a total of 1, 523, 238 heads of cattle. Cattle slaughter per year is estimated to be 62, 489 producing 29, 733 tonnes of meat. *Per capita* beef consumption per year in Tanzania is 11 Kg while that recommended by FAO is 50

Kg (MLD, 2006). Most cattle slaughtered and consumed in Arusha municipality originate from Meserani, Oldonyosambu and Lokii livestock markets. Some animals come from the neighbouring regions including Dodoma, Manyara and Mara. At Arusha Meat Company Abattoir, meat inspection is conducted by meat inspectors and approved carcasses are taken to meat shops in town. Consumers purchase beef from beef shops either for home consumption or for resale as ready-to-eat beef in form of *nyamachoma* or *mishikaki* in groceries and bars.

There are various scenarios which contribute to risk of contamination with thermophilic *Campylobacter* in *nyamachoma* and *mishikaki*. Food safety risk assessment has not been done to determine the safety of *nyamachoma* and *mishikaki* in Arusha, therefore, understanding risk assessment of thermophilic *Campylobacter* in ready-to-eat beef in this Municipality will address this gap in knowledge and have implications for design of risk management. The present study was conducted to establish the presence of contamination of beef sold in Arusha and associated food safety risks for consumers of beef products in informal outlets.

1.3 Objectives

1.3.1 Overall objective

To carry out food safety risk assessment of thermophillic *Campylobacter* of beef in Arusha Municipality.

1.3.2. Specific objectives

i) To estimate the risk of developing campylobacteriosis due to the consumption of ready to eat meat sold at informal markets (grilled meat "*nyamachoma*" pubs and skewer meat "*mishikaki*" sellers) in Arusha municipality.

ii) To assess knowledge, attitudes and practices of beef consumers on meat hygiene and food safety using participatory risk analysis approach.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview

In many respects, the potential for achieving food safety is greater in high income countries than elsewhere in the world. The food system is more integrated with high proportion of food being retailed through supermarket chains. Because both processors and supermarkets tend to operate on a large scale, they can afford the finance and human resources to put up policies to regulate food production. In this system, the consumers can afford to pay the cost of food hygiene services (WHO, 2002).

In large towns in Africa, most foodstuffs are purchased in markets and retail shops. However, the food chain is frequently long and complex. Fragmented food systems develop which involve numerous intermediates. Food processing is often carried out by small enterprises operating in substandard premises. Poor standards of food hygiene are therefore common. This is especially true of street vendors who prepare cooked foods (WHO, 2005). It seems sensible therefore to concentrate regulatory efforts on minimising the likelihood of deliberate food adulteration and the sale of obviously contaminated food. In rural areas of low income countries like Tanzania, where a large proportion of the world's population lives, the food system tends to be simple. Most people tend to grow their own food or buy locally-grown products, and nearly all meals are prepared at home.

2.2. Risk Analysis

Risk analysis is a systematic approach aimed towards assessing the likelihood of an adverse effect of an agent and suggesting intervention strategies. Is a function of both the probability of something undesirable happening and the magnitude of the impact of that hazard. Is a systematic way of gathering, evaluating and recording information leading to recommendations for a position or action in response to the hazard identified. Has three components (Fig. 1) namely, Risk assessment, Risk Management and Risk Communication (CAC, 1997).

2.2.1 Risk assessment

The Codex *Alimentarius* risk assessment model is appropriate tool for conducting microbiological risk assessment. The model has four components namely:

a) Hazard identification deals with qualitative evaluation of risk issues, identification of known health effects, cause-effect and addresses what agent present and capable of causing adverse health effect.

b) Hazard characterization is qualitative and/or quantitative evaluation of the nature of the adverse effects associated with presence of the agent in the food. It focuses on the dose-response relationship.

c) Exposure assessment looks on the degree of intake likely to occur (number of servings).

d) Risk characterization combines the previous steps in order to give an estimate of negative impacts due to a hazard and their likelihood of occuring.

2.2.2 Risk management

Risk management entails identifying, evaluating, selecting and implementing specific management measures to mitigate potential risks. The risk analyst identifies risk and may propose alternatives. Decision on preventive measures belongs to the public health policy maker and politicians in local state or national government.

2.2.3 Risk communication

The purpose of risk communication is to translate scientific information into messages that help the public put risks into perspective and make decision about such risks. Successful risk communication means that the message is understood by the target audience.

Risk-based approaches brought new insights and are now standard for food safety issues in developed countries as well as being the basis of rules governing international trade in food products (FAO/WHO, 1995).

Figure 1: Link between risk assessment, risk management and risk communication.

2.3 Food-borne and zoonotic diseases

The evaluation of food safety standards is driven by our increasing understanding of the burden imposed by food-borne and zoonotic diseases. This is responsible for an estimated 1.5 billion annual illness globally (Flint, 2005). Approximately 70% of deaths among under five children are linked to biologically contaminated food and water (Unnevehr and Hirschorn, 2000).

Impacts of food-borne diseases also include fatalities in other vulnerable groups (eg. elderly and people living with HIV/AIDS) and in 2-3 % of cases, severe and disabling long term effects such as joint disease, kidney failure, cardiac, retinal and neurological disorders. The latter chronic sequel of which many policy makers are unaware, probably represent a greater health and economic burden than the acute disease (Lindsay, 1997).

Urbanization, globalization, technological change and agricultural intensification are changing the domestic markets for livestock products on which the poor depend (Delgado *et al.*, 1999). The control of meat hygiene in markets and in food serving establishments is a necessary part of general food safety control measures, the object of which is to ensure that the consumer obtains food which is pure, fresh and unadulterated at the time of sale (Koch, 1957). Pathogenic bacteria may inhabit beef either before the animal is brought for slaughter at the abattoir or during slaughtering, transportation, and sale at meat shops (Neethling, 1999). The water used for cleaning and washing may act as a vehicle of transmission of the bacteria.

2.4 Incidence of Pathogenic enterobacteria in food in Tanzania

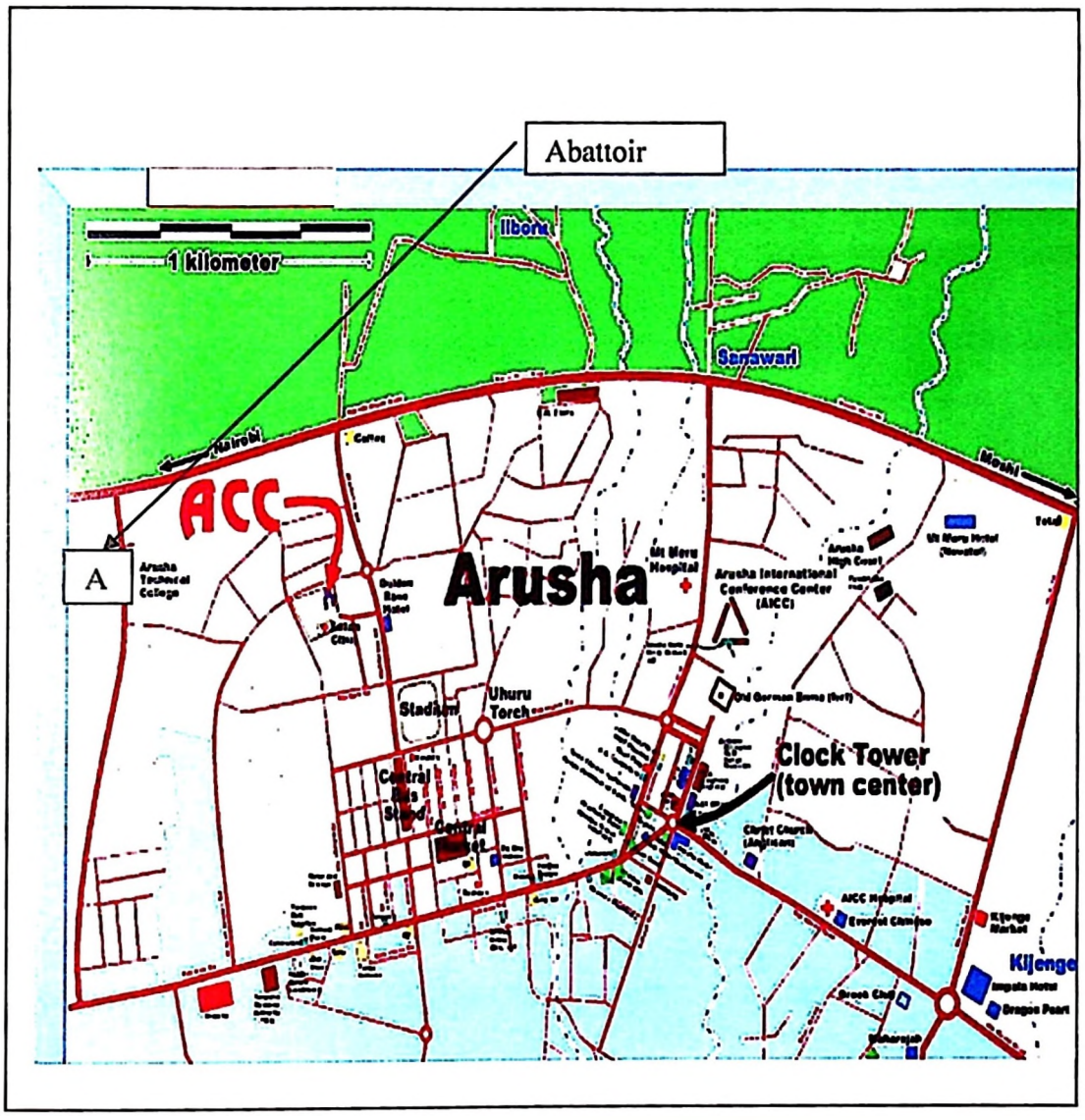
Salmonella typhimurium and *S. enteritidis* have been isolated from chickens (Minga *et al.*, 1988). Lindblom *et al.*,(1995) reported that *Campylobacter jejuni, C. coli* and enterotoxigenic *Escherichia coli* were important causes of diarrhoea in children in Tanzania and reported that 18% and 20% of them were infected with *Campylobacter* and *E. Coli* respectively.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was conducted in Arusha municipality (Plate 1). northern part of Tanzania. Arusha municipality is situated at latitude 3°22` to 3°367`S and longitude 36°41` to 36°683` with an elevation of 1265 meters above sea level. Arusha region has a population of 1, 288. 088 people with 286. 241 households. The population of Arusha Municipality is 341. 136 (URT. 2002) growing at a rate of 4% per annum. Arusha has a modern abattoir which is the main slaughter house for cattle in the municipality.



Automobiles and supplies

North wards

Building supplies

Church, church-related

Food (grocers, butchers)

Government, public

\+ Hospitals

Parking

Petrol station

Hotel or restaurant

South wards

Plate 1: Map of Arusha Municipality showing study area

3.2 Study Design

This was a cross-sectional study design in which Participatory Risk Assessment methods were used. The study was carried out through interaction between practitioner and participants in which available information on attitude, knowledge and perception on beef handling practices were discussed by members of the community to reach specific best-bet solutions (Catley, 1999). Since the method involves interaction between practitioner and participants, proper knowledge of communication skills and correct use of the specific method are very important. In this approach, stakeholders in informal beef markets (butcher sellers, customers for household consumption, *nyamachoma* and *mishikaki* sellers) were involved in the discussion (Focus Group Discussion). Key questions were asked to the participants and answers were left to them to come to a conclusion. A diagram (risk pathway) (fig. 2) was designed to map the risk of thermophilic *Campylobacter* to consumers by examining the risk pathway between the point of sale and consumption. The pathway identified potential points where microbial contamination and recontamination could occur from reception of beef at the butcher shop to consumption.

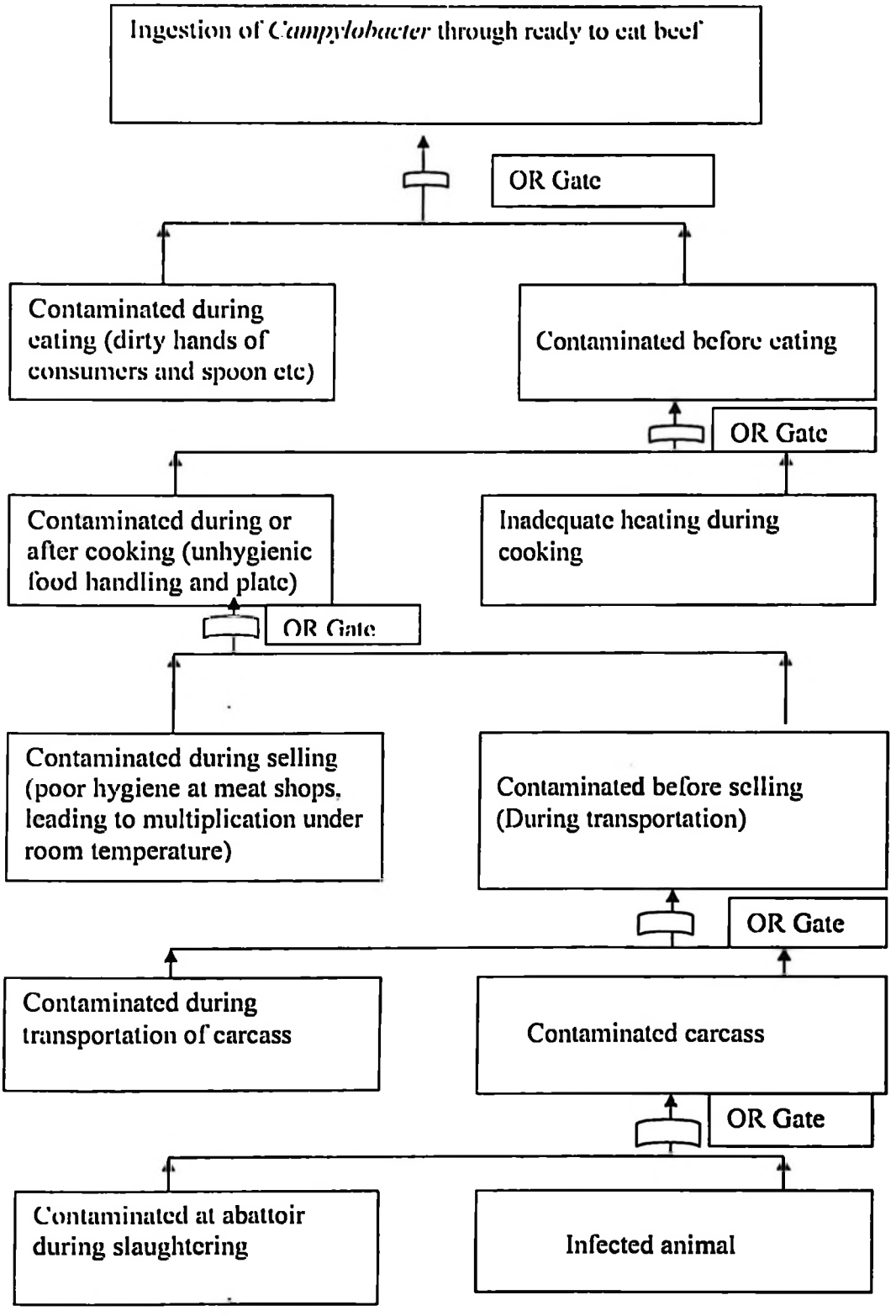


Figure 2: Fault tree diagram for ready to eat beef contamination with *Campylobacter* in Arusha

3.3 Sampling Design

Random sampling of meat shops was conducted. A list of meat shops in Arusha Municipality was obtained from the Municipal Veterinary Office. Each shop was assigned a number. The numbers to be selected were generated using a random number generator and shops were selected to satisfy the desired sample size.

3.4 Sample Size Determination

Sample size was calculated using the formula developed by Martin *et al.*, 1987:-

$n = Z\alpha^2 \times P\,(1-P)/d^2$.

Where n= required sample size. $Z\alpha$ = is the Z value for a given level of confidence ; P= is a known or estimated prevalence; 1-P = the probability of having no hazard; d= allowable error of estimation: For the purpose of this study a confidence level at 95% with an allowable error of estimation of 5%: Estimated prevalence of Thermophilic *Campylobacter* from previous study was 9.3% (Mdegela *et al.*, 2006).

$$n = \frac{1.96^2 \times 0.093\,(1-0.093)}{0.05^2} = 129.6171$$

The calculated sample size was 130 samples however, the sample size was arbitrarily approximated to 160 samples of surface swabs and 1 gram pieces of ready-to-eat beef.

3.5 Isolation of Thermophilic Campylobacter

From each carcass, one swab per sampling area was collected (i.e. four swabs per carcass namely neck, ribs, ham and thigh). A 25 cm^2 area on carcass surfaces was swabbed using sterile cotton wool swabs soaked in normal saline. After sample collection each entire swab was placed in sterile universal bottles containing 10ml of

Preston broth (Oxoid Ltd. Basingstoke. UK) with Preston supplements for enrichment and stored in cool box with ice pack at 4^{0}C. The samples were subsequently sent to the laboratory at the Veterinary Investigation Centre (VIC) Arusha and analyzed within 4 hours from the time of collection. Isolation and identification of zoonotic *Campylobacter* was carried out according to the method described by Karmali *et al.* (1986). The samples in the Preston broth were loaded in the micro-aerophilic candle jars (Coldstream Engineering Ltd. Arista, Sweden) with a lighting candle and incubated at 37°C for 24 hours as described by Skirrow and Benjamin (1980). After incubation, the universal bottles with enriched samples were slowly agitated and sub-cultured onto modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid Ltd. Basingstoke. UK) for primary isolation of thermophilic *Campylobacter*. The inoculated petri dishes were incubated at 43°C for 48 hours under microaerophilic conditions as described by Skirrow and Benjamin (1980) and slender like colonies (resembling *Campylobacter*) were sub-cultured onto blood agar (Oxoid Ltd. Basingstoke. UK) at 43°C under micro-aerophilic conditions for 24 hours.

Suspected thermophilic *Campylobacter* colonies on blood agar that were Gram-negative, curved or spiral rods and showed corkscrew like motion, positive to catalase, oxidase and nitrate reduction tests were further tested for hippurate hydrolysis, H_2S production and susceptibility to nalidixic acid and cephalothin. These parameters formed the basis for the identification of *C. jejuni, C. coli* or *C. lari,* as described by On (1996). For the hippurate hydrolysis test, those organisms yielding a positive test were considered *C. jejuni*, while those organisms that showed a negative reaction were considered *C. coli* and *C. lari*.

Nalidixic acid resistance test (NART)

Campylobacter suspected colonies which were negative to hippurate hydrolysis test were streaked on blood agar (Oxoid® Ltd., Basingstoke, Hampshire, England) supplemented with 10% horse blood. Susceptibility tests to nalidixic acid (30µg) (Oxoid. UK) and cephalothin (30µg) (Oxoid® Ltd. Basingstoke, Hampshire, England) were performed in accordance with the criteria set by the National Committee for Clinical Laboratory Standards (NCCLs) using the disc diffusion method (NCCLs 2002). A loopful of *Campylobacter* colonies was uniformly streaked on the blood agar and antibiotic discs were distributed over the inoculated plates using a sterile forceps. The plates were placed in an incubator and incubated at 37°C for 24 hours under microaerophilic condition in Skirrow's protocol and hydrogen-enriched atmosphere in Cape Town protocol. The isolates were classified as sensitive and/or resistant according to the standardized tables supplied by the (NCCLs 2002). *Campylobacter* strains that were resistant to both drugs were considered *C. lari* (On. 1996).

Nitrate reduction

The isolates which were susceptible for NART were subjected to nitrate reduction test for confirmation of *C. coli*. About 0.3 ml of sterile normal saline was added in a plastic bijou bottle containing nitrate (Lipfilchem s.r.l. Roseto, d. A. (TE)-Italy) reagent and mixed with a loopful of *Campylobacter* isolates and incubated (Memmert, Germany) at 37°C for 24 hours. A drop of *alpha-naphtylamine* and one drop of sulfanilic acid were added to the mixture and mixed gently for a few minutes. The formation of red orange colouration confirmed *C. coli*.

3.6 Focus Group Discussion

A checklist of key questions was prepared and focus group discussions were conducted. The focus group discussions (one in each study ward), were conducted for consumers only as it was very difficult to conduct focus group discussion with the beef sellers because of their tight schedule.

The discussions were based on aspects of knowledge on microbial contamination in general, attitude and perception on beef markets as well as handling practices for meat. A sound recording device (Sony Digital Camera, Made in Japan) was used to record the discussion and thereafter, information was transcribed into Microsoft word document. For confidentiality purposes the information was deleted from the device.

3.7 Questionnaire Survey

The structured questionnaires were administered by personal interview to beef consumers at the time when they came to buy beef at the butchers. Once they had purchased beef, the owner/seller introduced the customer to recorder and if one agreed to participate then he/she was interviewed. A total of 140 customers, 35 butcher shopkeepers and 25 *nyamachoma* and *mishikaki* sellers were interviewed.

3.8 Exposure Assessment

The exposure to "ready-to-eat" meat contaminated with *Campylobacter* was estimated by stratifying the exposure sources into five categories: two categories of type of sellers (Roast meat *nyamachoma* pubs and Skewer meat *mishikaki* sellers)

and three categories of geographical zones of Arusha Municipality (North, Central and South). This was done because the quantities of consumption per person, numbers of customers and mode of sales were different between *nyamachoma* pubs and *mishikaki* sellers, and the hygiene status of *nyamachoma* pubs was different between Northern and Central zones which were more hygienic and Southern which were observed to be mostly unhygienic.

For the exposure assessments, the variables were modeled stochastically using listed distributions or simulations (Table 1). The daily quantities sold (DQcont) and proportions (Pcont) of ready-to-eat meat contaminated with *Campylobacter* were then calculated separately for *nyamachoma* and *mishikaki* using the following formula:-

DQcont = $\sum$ (number of sellers x number of customers x average consumption per person x contamination rate)

*$\sum$- the sum of the calculation of North, Central and South

Pcont = DQcont / (total number of sellers x number of customers x average consumption per person).

Table 1: Variables modeled for the exposure assessment in Arusha municipality, Tanzania from January to March 2010.

Variables	Distribution or simulation	Source of data
Number of *nyamachoma* pubs and *mishikaki* sellers in each zone	Deterministic	Arusha Municipal Business Office
Average number of customers a day	Average of bootstrap	Interviews with sellers
Average quantity of consumption per person	Average of bootstrap of quantity sold /number of customers a day	Interviews with sellers
Contamination rate	Beta distribution	Microbiological tests

Each zone means each of North, Central and South. The contamination rate in Central was estimated using the results in North.

3.9 Hazard Characterization

Parameters (alpha and beta) for Beta Poisson dose-response model for *Campylobacter* infection were calculated using statistical function of Excel using the results presented by Black (1988) in Most Likelihood Estimation. Under the assumptions that 1) customers at *nyamachoma* pubs and *mishikaki* sellers are over 5 years old, 2) illness occurs when infection is established in susceptible individuals, and 3) illness in naturally immunized population occurs with the same probability among naturally immunized population as in Uganda. The dose-dependent probability of illness (P-illness) in the present study was calculated as below. The population data was obtained from the age-population pyramid of Uganda (UMoH, 2006).

P-illness = probability of illness calculated using alpha and beta x (incidence under 5/ population under 5)/(incidence over and equal to 5/population over and equal to 5) The dose used for the calculation of P-illness was modelled with bootstrap of three low dose (cfu/g) variables presented in the two studies (0.1 in cattle carcasses in USA (McNamara, 1995) and 0.29 in beef and 0.29 in unweaned veal in New Zealand (Wong, 2007), as the microbiological test in the present study did not test for cfu/g. These doses were selected among published data as the contamination in such informal pubs was assumed to occur with a low but non-negligible concentration of *Campylobacter*.

3.10 Risk Characterization

The risk of illness due to consumption of ready to eat meat purchased from informal sellers (*nyamachoma* pubs and *mishikaki* sellers) was estimated as an incidence rate: daily incidence per 1000 people by dividing daily total incidence (TI) estimated by daily total number of customers (TNC) in Arusha.

TI = $^{*}\sum$ (Number of shops x number of customers x contamination rate x P-illness)

TNC = $^{*}\sum$ (Number of shops x number of customers)

*$\sum$- the sum of the calculation of six categories: *nyamachoma* and *mishikaki* at North, Central and South

For exposure assessment, hazard characterization and risk characterization, Monte Carlo simulation was run for 10.000 iterations using @Risk (Palisade) software.

3.11 Sensitivity Analysis

To examine the sensitivity of risk inputs, sensitivity analysis was run for 63 simulations and 1000 iterations using @Risk (Palisade).

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation of thermophilic Campylobacter in raw beef, nyamachoma and mishikaki

Table 2 shows the isolation rate of thermophilic *campylobacter* from raw beef, *nyamachoma* and *mishikaki* obtained in different informal selling outlets in Arusha municipality. Worthy is the higher *Campylobacter* isolation rate in almost all products in the southern ward.

Table 2: Isolation rate of thermophilic *Campylobacter*

Type of Product	Ward	No. Sampled	No. positive	Probability
Raw beef	Northern	41	1	0.024
	Southern	32	8	0.25
Nyamachoma	Northern	21	1	0.05
	Southern	24	7	0.316
Mishikaki	Northern	20	3	0.15
	Southern	20	7	0.35

4.2 Exposure Assessment

Table 3 shows quantities, consumption rate and probability of eating contaminated nyamachoma and *mishikaki* in Arusha Municipality. Results revealed higher probabilities of contamination in *mishikaki* than in *nyamachoma.*

Table 3: Exposure assessment of thermophilic *campylobacter* resulted from *nyamachoma* and *mishikaki* in Arusha Municipality.

Parameter	Result		
	Mean	5%	95%
Total quantity of *nyamachoma* contaminated	3595 Kg	1745 Kg	6133 Kg
Total quantity of *nyamachoma* consumed per day	23152 Kg	16811 Kg	30049 Kg
Probability of eating contaminated *nyamachoma*	15.5%	8.3	24.9
Total quantity of *mishikaki* contaminated	165	57.3	327
Total quantity of *mishikaki* consumed per day	474	167	837
Probability of eating contaminated *mishikaki*	34.7%	21.3%	49.1%

4.3 Hazard Characterization

In order to establish hazard characterization for thermophilic campylobacter in this study, the following assumptions were made: - (i) customers at *nyamachoma* pubs and *mishikaki* sellers are over 5 years old. ii) illness occurs when infection is established in a susceptible individual iii) illness in naturally immunized population occurs with the same probability among naturally immunized population. Using @Risk (Palisade) software at ten thousand iterations, the parameters for dose-response relationship of infection were 0.145322 for alpha and 7.589651 for beta. The probability that naturally immunized person develops illness when he/she ingests enough dose to develop illness for a susceptible person was 0.131.

4.4 Risk Characterization

For risk characterization, Monte Carlo simulation was run for ten thousand iterations using @Risk (Palisade) software and resulted to an estimated incidence rate of campylobacteriosis in Arusha municipality per day to be 6.43 people (90% CI: 3.4-10.4) per 1000 people.

4.5 Sensitivity Analysis

To examine for sensitivity of risk inputs, sensitivity analysis was run for 63 simulations and one thousand iterations using @Risk (Palisade) software and direct estimation was done by using Beta Poisson dose-response model (Fig. 3). Tornado graphs indicate estimation points within the value chain where contamination could probably occur. The estimation point parameters which contributed significantly to the probability of eating contaminated ready-to-eat beef were initial population of *Campylobacter* in raw beef at butcher level, contamination of roast and skewer beef at the kitchen and quantity of roast and skewer beef consumed per person. Sensitivity analysis simulates the risk scenarios in different value chain to real life situation. Worthy is the high value of initial population of *Campylobacter spp* (Pinf nyama B31) in raw beef; however in this study we did not perform quantification tests to find colony forming units per gram.

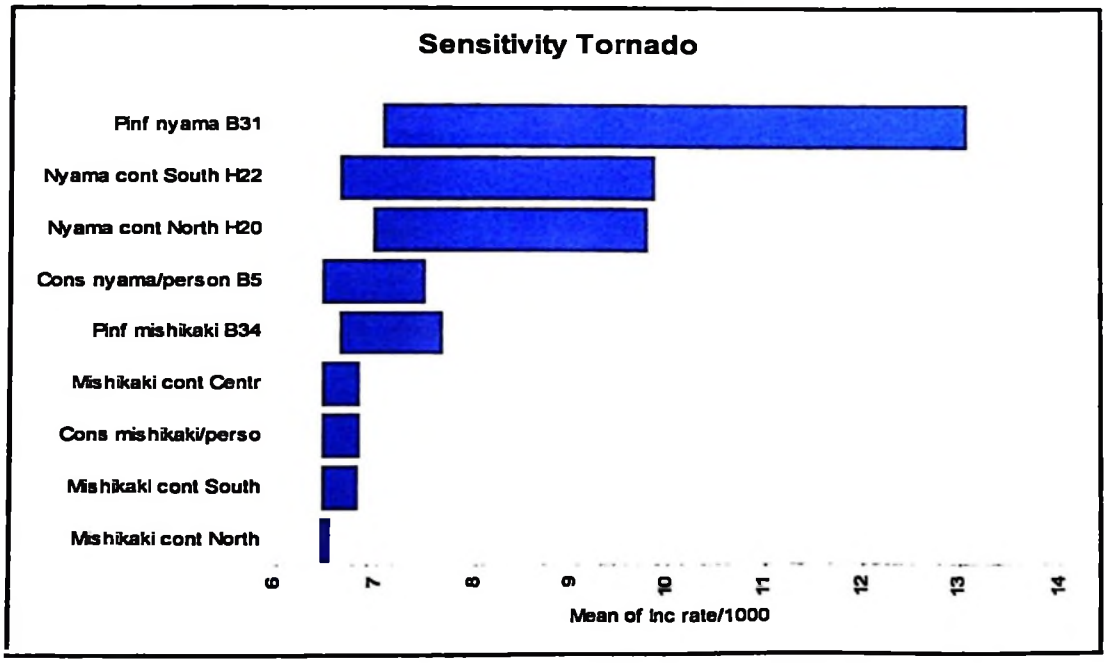


Figure 3: Tornado graphs of sensitivity analysis obtained by Monte Carlo simulation

4.6 Results of Questionnaire Survey

The tables 4 indicate responses from beef customers interviewed at various butcher shops in Arusha municipality. The responses describe factors that could contribute to increase or decrease risk of consuming contaminated beef. Seventy eight percent (78%) respondents preferred to eat beef because it is nutritious and that the food safety criteria for choosing to purchase in a particular shop was good hygiene. A large proportion of respondents (63.8%) bought beef three times a week and the majority (85.4%) purchased between 0.25 and 1Kg. Forty nine percent (49 %) purchased beef in the morning and a large number of respondents (68%) graded beef by looking at fat content and colour. Sixty percent (60%) respondents served food within 30 minutes after cooking. but nobody mentioned campylobacteriosis as one of the risk which may result from consumption of beef. Rift valley fever was mentioned by fifty two percent (52%) of the respondents followed by gout. Seventy five percent of respondents had not received training on food hygiene however, the hygienic measure taken to ensure food safety was eating food while is still hot. Washing hands with soap and water was not common.

Table 4: Responses of consumers to factors which could contribute to increase or decrease of risk of consuming contaminated beef in Arusha Municipality

Parameter	Response (n= 140)	Frequency (%)
Beef preference	Beef is safe	10.2
	Beef is cheap	9.5
	Good taste	17.5
	Interest	21.9
	Tradition	18.2
	Nutritious	22.1
Reasons for purchasing beef at a particular butchershop	Good hygiene	59.1
	Quality beef is sold	29.9
	Low price	11.0
Frequency of buying beef per week	Once/week	9.4
	Twice/week	26.8
	Thrice/week	63.8
Amount of beef purchased per day	0.25-1Kg	85.4
	1.25-2Kg	9.5
	Above 2Kg	5.1
Time of purchasing beef	Morning	48.9
	Afternoon	9.5
	Anytime	41.6
Quality assessment of beef	Fat content	36.5
	Light reddish colour	31.6
	Inspection stamp	16.7
	Carcass freshness	15.2
History of illness	Yes	2.2
	No	97.8
Washing beef	Yes	94.9
	No	5.1
Source of water used	Tape water	91.2
	Other source	8.8
Time taken for beef to be cooked properly	5-30 min	46.6
	31 - 60 min	33.5
	Above 60 min	14.9
	Do not know	5.0
Time from cooking to eating	0-30 min	51.4
	31-60 min	18.1
	Above 60 min	7.2
	Do not know	23.3
Knowledge on risk of beef consumption	Yes	52.6
	No	47.4
Type of risks mentioned	Rift valley fever	36.9
	Gout	43.1
	Allergies	12.3
	Helminthosis	7.7
Training on food hygiene	Yes	24.8
	No	75.2
Length of training	1week-1Month	29.4
	2months-6months	32.4
	Above 6months	38.2
Hygienic measures taken to ensure food safety.	Eating food while hot	40.8
	Cover food	20.4
	General hygiene	30.1
	Wash hands with soap and water	8.7

CHAPTER FIVE

5.0 DISCUSSION

Results of this study have demonstrated that Thermophilic *Campylobacter* was isolated in all products in Arusha municipality however. Southern wards had recorded a higher frequency in all products suggesting the risk of contracting campylobacteriosis is greater in those wards than in the Northern wards. The isolation rate of *Campylobacter* in beef carcasses in butcher shops found in this study is comparable with other studies where thermophilic *Campylobacter spp* isolated from the cattle carcasses (Ono and Yamamoto, 1999; Beach *et al.*, 2002; Hakkinen *et al.*, 2007; Valnegri *et al.*, 2008).

Lower isolation rate were reported by Nonga *et al.*, 2009 and Tefera *et al.*, 2008 who recorded the contamination rate of 9.3% and 10.1% respectively. Isolation of thermophilic *Campylobacter* in butcher shops suggests there is contamination of carcasses at the abattoir or during transportation of meat, at the butcher shop or during beef preparation (Appendix 2). Under normal circumstances, *Campylobacter* are enteric microorganisms and stay in the intestinal contents. Obviously cross-contamination can originate from the feacal materials of the same animal or different animals in the slaughter house environment or equipment especially during flaying, evisceration or from cross contamination from hide to carcass (Gannon 1999, Hakkinnen *et al.*, 2007). This is also supported by observation seen at butcher shops where offals are sold mixed with other carcass parts (Appendix 1).

The probabilities of consuming beef contaminated with thermophilic *Campylobacter* in *Nyamachoma* pubs and *Mishikaki* selling points in Arusha municipality were higher than those reported in Wales (Meldrum and Ribeiro, 2002). These researchers

found that in ready-to-eat beef the risk was zero. The probability associated *nyamachoma* in this study is low when compared to results described by Rao *et al.*, (2001) who noted a probability of 0.17 in Sweden. However, probability of *Campylobacter* observed in *mishikaki* is higher when compared to that described by Rao *et al.*, (2001). Probabilities described by this study in both *Nyamachoma* and *Mishikaki* highlights the risk of consumption of contaminated ready to eat beef and/or cross contamination. Probability of contaminated *Mishikaki* is significantly higher than that of *nyamachoma* probably due to the fact that *mishikaki* have large surface area than *nyamachoma* making it easier to be contaminated (Appendices 3 & 4). Alternatively, the beef sold to consumers was not properly reheated. In their study, Francina and Alexander, (1997) reported the presence of *Campylobacter* in meat kept on display for 3.5 hours.

The difference in the quantities of *nyamachoma* and *mishikaki* sold per day is most likely due to the relative difference in number of *Nyamachoma* pubs and *mishikaki* places. As per business census, (2002) Arusha had more *nyamachoma* centres (816) than *mishikaki* places (279). The method used to sell *nyamachoma* is different from the one used for *mishikaki*. *Nyamachoma* is sold at a minimum of 500g where as in *mishikaki* the minimum is one piece of *mshikaki* that is approximately 50 g. The parameters for dose-response relationship of infection were 0.145322 for alpha and 7.589651 for beta. These values are similar to those reported by Teunis, (2000). However in some major risk studies reported for thermophilic *Campylobacter* the dose-response is not well characterized (Lailai *et al.*, 2006). The maximum likelihood estimates for the beta Poisson model parameters was also reported to be 0.145 and 7.59 for alpha and beta respectively (Black, 1988).

The probability that a naturally immunized person develops illness when he/she ingests enough doses to develop illness for a susceptible person was 0.131. Arusha municipal has 341,136 people and therefore approximately 44,689 people could develop campylobacteriosis if they ingest a sufficient dose. The estimates above are lower when compared to similar public health outcome for beef products estimated in the United States where it was estimated 152.000 people developed campylobacteriosis (Anderson *et al.*, 2001). This is probably attributed to the poor hygienic conditions prevailing in ready-to-eat meat premises in developing countries suggesting high contamination of meat.

Incidence rate of campylobacteriosis in Arusha per day was estimated as 6.43 people per 1000 people. This is equivalent to 2, 194 people when related to the total population density in Arusha municipality. This can also be expressed as 30/100,000. This population rate is lower when compared to the magnitude of annual incidence of 50/100000 in population in the United Kingdom (Richard and Taylor, 2000). However, the overall incidence rate for thermophilic *Campylobacter spp* in the European Union countries is 47.6 cases in 100,000 population in which prevalence in bovine meat was 11.9 percent whereas that reported in Uganda is 73.7 cases in 100, 000 people (UMoH, 2006).

Campylobacter isolation rate from humans in developing countries ranges from 5 to 20 % (Richard and Taylor, 2000). Despite the lack of incidence data from national case-control community based studies have provided estimates of 40,000 to 60,000/100,000 for children<5 years of age (Rao *et al.*, 2001). In contrast the figure in developed countries is 300/100,000 (Tauxe, 1992). Estimates in the general

population in the developing and developed countries are similar, approximately 90/100.000 (Taylor, 1992). A study in Tanzania reported the isolation rate of *Campylobacter spp* from diarrhoea specimens from <5 years old to be 18% (Megraud *et al.*, 1990).

In this study we did not determine concentration of campylobacter in the sample studied. Robinson, (1981) reported that a dose of 500 cells of *Campylobacter spp* are sufficient to cause illness. Usually symptoms show between 2 to 7 days after ingestion of contaminated food or water.

During questionnaire survey, most of the respondents indicated they preferred to eat beef because it is nutritious and that good hygiene was the criteria for decision to buy beef in a particular butcher shop. Such opinions were put forward probably due to the fact that most of respondents had primary school education. A large proportion of respondents purchased beef three times in a week and beef were bought in the morning. The grading method for most of the respondents was looking on fat coverage. This is the most commonly used method to evaluate beef quality because it is cheap, time saving and easy to do (Bratzler, 1932). Fourty seven percent respondents reported to cook beef for 5 to 30 minutes and consume it within 30 minutes after cooking. The proper beef cooking times and the correct beef cooking temperatures are extremely important in avoiding food-borne illness that may arise from undercooked food. Fifty three percent of the respondents had some knowledge on risks that might arise as a result of beef consumption. The main risk reported was gout. Gout is caused by high levels of uric acid in blood. The main predisposing factors are family history, obesity, excessive alcohol intake and high

purine diet (Nakanishi *et al.*, 1999). The study showed that the seventy five percent of respondents had not received training on food hygiene. This suggests the link between lack of training and *Campylobacter* contamination rate in beef in Arusha. The main control measure taken by most of respondents was to eat food while still hot.

Cattle slaughtered at Arusha meat abattoir originate from Meserani, Ngaramtoni and Rokii livestock markets. Cattle also come from neighbouring regions which include Manyara and Dodoma (Kondoa district). Cattle traders buy cattle from these livestock markets and send for slaughter to Arusha Meat Company abattoir. Meat sellers buy beef carcasses at the abattoir from cattle traders. The *Nyamachoma*, *mishikaki* sellers and other customers purchase beef from the butcher shops. This marketing chain suggests that if hygiene and inspection are monitored and complied, risk of *Campylobacter* contamination could be reduced.

CHAPTER SIX

6.0 CONCLUSSION AND RECOMMENDATIONS

The present study, like other studies elsewhere, clearly shows that there is a high probability of contamination of beef with *Campylobacter*. This has a higher impact and poses a serious health risk to beef consumers.

Nyamachoma and *mishikaki* are widely consumed in the country increasing the likelihood of *Campylobacter spp* transmission to humans if meat is not prepared well. Usually *nyamachoma* and *mishikaki* are sold at bars, groceries and take away restaurants. Nowadays the number of bars and groceries in Tanzania are increasing suggesting human exposure to hazards contained in ready to eat beef. There must be a mechanism of ensuring food safety at these selling outlets. Contamination of carcasses with *Campylobacter spp* has indicated the need to apply good hygienic standards at all levels from farm to table in order to ensure food wholesomeness and safety in beef.

Awareness of the importance of good hygienic practice in the butcher shop is very important in order to reduce contamination in beef carcasses. This should include proper offloading of beef from meat van, hanging, cutting, storage and dispatch at sale. Butcher shops should be constructed to prevent flies, dusts and other contaminants.

All sellers who are in contact with beef should undergo regular medical check up to reduce chance of human to human infection via beef. Proper cooking and handling of beef are important in preventing *Campylobacter* infections; food should be served

while still hot to prevent recontamination. Hand washing with warm water before eating reduces risk of contamination.

Local governments should review and implement existing national policies and regulations pertaining beef production, marketing, inspection as well as hygiene practices. Generally public education on good hygienic practice regarding food consumption is the best risk mitigation procedure to ensure and safeguard health of consumers.

Because of the increasing incidence, expanding spectrum of infections, potential of HIV-related deaths due to *Campylobacter*, interest in campylobacteriosis research and control should be emphasized. National surveillance programmes and international collaborations are needed to address the substantial gaps in the knowledge about the epidemiology of campylobacteriosis in Tanzania.

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APPENDICES

Appendix 1: A picture of butchershop indicating intestines being sold with other parts of the carcass predisposing contamination of *Campylobacter* in Arusha municipality, Tanzania, January-March 2010. (Picture taken by the author)

Appendix 2: Picture of *nyamachoma* centre in Arusha, Tanzania showing beef preparation. (Picture taken by the author)

Appendix 3: A picture of mishikaki being grilled on charcoal cooker in Arusha, Tanzania, January –March 2010. (Picture taken by the author)

Appendix 4: A picture of ready-to-eat *Nyamachoma* in Arusha, Tanzania.

(Picture taken by the author)