

**PREDISPOSING FACTORS, ISOLATION, SENSITIVITY TO ANTIBIOTICS
AND CONTROL METHODS OF SALMONELLOSIS IN NAKURU NORTH SUB-
COUNTY, KENYA**

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DECLARATION

This thesis is my original work and has not been presented for a degree or other awards in any other University.

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Declaration by supervisors

This thesis has been submitted to graduate school with our approval as university supervisors

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DEDICATION

To my loving parents, Mr. and Mrs. Waitathu who taught me from an early age that dreams, hopes and intentions are nothing without actions.

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ABBREVIATIONS AND ACRONYMS

API	Analytical profile index
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
Hrs	Hours
ISO	International Standard Organization
KFSSG	Kenya Food Security Steering Group
km	Kilometres
NTU	Nephelometric Turbidity Units
PET	Poly Ethylene Terephthalate
ROSA	Result-oriented sanitation concepts in peri-urban areas in Africa
SODIS	Solar disinfection
Spp	Species
Sq.	Square
TSI	Triple Sugar Iron
UV	Ultraviolet rays
WHO	World Health Organization
WS	Water and sanitation
XLD	Xylose lysine desoxycholate agar

ABSTRACT

Salmonellosis is one of the most common and widely distributed group of enteric diseases in the world today. It causes high mortality and morbidity especially in developing countries. This study was aimed at identifying the factors that predispose people to salmonellosis. In addition the study was meant to isolate *Salmonella*, and determine its antimicrobial sensitivity and test the effectiveness of water treatment for the control of salmonellosis. To identify the predisposing factors of salmonellosis, a questionnaire was used. For the isolation of *Salmonella*, a total of 630 samples were collected from raw cows' milk, sheep and cattle intestinal wastes, raw fruit and vegetable salads, waste water, water sources and water that had been treated by the study population through boiling, chlorination and filtration. Samples were also collected from water that was treated through solar disinfection. The samples were pre-enriched using peptone water then selectively enriched using Selenite F broth and incubated at 37 °C for 24 hrs and subcultured in xylose lysine desoxycholate (XLD) agar and *Salmonella-Shigella* agar in five replicates. Typical *Salmonella* colonies were confirmed by biochemical test using API E-20 and the species serotyped. The isolated serovars were tested against seven antibiotics; cephalexin, nalidixic acid, chloramphenicol, ciprofloxacin, gentamicin, amoxicillin and sulfa-trimethoprim. The results were analyzed by the use of chi-square test, correlation test and Anova using Statistical Package for Social Sciences (SPSS version 11.50) software. Level of education, occupation, method of food storage, cleaning of kitchen utensils, hand washing, human waste disposal, animal wastes, presence or absence of sewers, waste water, tap water, river water and water treatment were significantly associated with salmonellosis while sex, well water and method of water treatment were not. There was no significant difference between the microbial load of *Salmonella* isolates from milk, sheep and cattle intestinal wastes, waste water, fruits and vegetable salads in Maili 5, Bahati and Kabatini. However, River Kandura's water mean *Salmonella* isolates varied significantly from upstream to downstream. Of the 105 *Salmonella* isolates *Salmonella enterica* serovar Typhimurium were (45.7 %), *S. enterica* serovar Typhi (22.9 %), *S. enterica* serovar Enteritidis (21.9 %) and *S. enterica* serovar Dublin (9.5 %). All the serovars were susceptible to gentamicin with a minimum of at least 2 *Salmonella* being resistant. All the samples that turned out positive were highly contaminated with *Salmonella* apart from isolates from the upstream of river Kandutura, the most effective method of water treatment being used in the study area was chlorination. Solar disinfection is effective upon continuous exposure of water to sunlight for 3 to 5 h. In addition, *Salmonella* isolates from Nakuru North Sub-County are sensitive to gentamicin. There is need to educate people on the predisposing factors of the disease, regular food inspection by the authorities concerned, sensitization of the entire population on the need of proper use of antimicrobials and use of gentamicin for the treatment of salmonellosis.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Salmonella are well-known pathogens, highly adaptive and capable of causing disease in humans and/or animals. *Salmonella* infections are capable of producing serious infections that are often food borne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and extra intestinal disease (Fluit, 2005). The main reservoirs for non-typhoidal *Salmonella* are animals such as poultry, livestock, pets and reptiles. *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi colonize only humans and are often acquired through feacally contaminated food or water, a person who has typhoid fever, or from chronic carriers (CIDRAP, 2006).

While some serovars of *Salmonella enterica* such as *Salmonella enterica* serovar Typhi, *S. enterica* serovar Paratyphi, *S. enterica* serovar Enteritidis and *S. enterica* serovar Derby cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host such as *Salmonella enterica* serovar Gallinarum in poultry and *Salmonella enterica* serovar Abortus-ovis in sheep. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhoea and vomiting, to typhoid fever, a life threatening infection (Hensel, 2004). The outcome of *Salmonella* infections is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, for the bacterium it is determined by virulence factors (Alphons *et al.*, 2005).

Serotypes adapted to man, such as *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi, usually cause severe diseases in humans as a septicaemic typhoidic syndrome (enteric fever). These serotypes are not usually pathogenic to animals. Serotypes that are highly adapted to animal hosts, such as *Salmonella enterica* serovar Gallinarum (poultry) or *Salmonella enterica* serovar Abortus-ovis (sheep) usually produce very mild symptoms in man (Fluit, 2005). However, *Salmonella enterica* serovar Choleraesuis which has the pig as a primary host also causes severe systemic illness. In the same way, *Salmonella enterica* serovar Dublin, which has a preference for bovines, is primarily responsible for the systemic form of salmonellosis in people. In young calves this disease causes high mortality, while in adult cattle it results in fever, reduced milk yield, diarrhoea, abortion and occasionally death. Ubiquitous serotypes, such as *Salmonella enterica* serovar Enteritidis or *Salmonella enterica* serovar Typhimurium, which affect both man and animals, generally cause gastrointestinal infections usually less severe than enteric fever. However, they also have the capacity to produce typhoid-like infections in mice and in humans, or asymptomatic intestinal colonization in chickens (Velge *et al.*, 2005).

Salmonellosis is one of the most emerging and re-emerging infectious diseases in the world (WHO, 2004). The high prevalence of salmonellosis is attributed to lack of adequate water supply, poor sewage effluent disposal coupled with contaminated foods (Jones, 2005). About 1,195 outbreaks of salmonellosis were reported in Brazil in 2007, with 22.6 % of them provoked by the consumption of foods with raw eggs (Wray, 2001). It is estimated that 22 million typhoid cases are reported with 200, 000 deaths in each

year. The estimates in Africa are very low because very few people seek medical attention. For example, 59/100, 000 in East Africa and 39/100, 000 cases in Kenya per year (Kariuki *et al.*, 2012). The highest burden due to salmonellosis is felt mostly in the developing countries (Henson, 2003).

1.2 Problem statement and justification

Salmonellosis is an important global health problem which causes substantial morbidity and thus it has a significant economic impact. In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food borne diseases are still prevalent and most important health problem in most countries (Dominguez *et al.*, 2002). Many foods, particularly those of animal origin, have been identified as vehicles of transmission of these pathogens to human beings and spreading them to the processing and kitchen environment (Uyttendaele *et al.*, 2002).

The major constraints for development in the food safety in Nakuru North Sub-County are the lack of basic infrastructure and the appropriate technology, and an acute shortage of trained personnel in the food safety and quality control. The food production system in Nakuru North Sub-County is still a livelihood enterprise, and commercial food producing companies are very few (KFSSG, 2008). Routine food inspection and quality control is a responsibility of public health officers under the Ministry of Health. A majority of the food inspectors are certificate holders with short-term training in food inspection (Moy and Schlundt, 2005). The laboratories are not fully equipped, both in terms of facilities as well as in manpower. Therefore, with the lack of resources such as skilled manpower and

laboratory facilities to efficiently carry out the regulatory works cases of salmonellosis have escalated over the recent past (Kariuki *et al.*, 2012).

In some instances, there have been incidences of *Salmonella* mass food poisoning such as in schools, picnics sites and villages in Nakuru North Sub-County, attributed to consumption of contaminated foods (ROSA, 2007). Although *Salmonella* in foods has been recognized as a major source of infection, no systematic studies have been carried out with respect to factors that predispose people to salmonellosis, *Salmonella* load of food and environmental samples collected from Nakuru North Sub-County, the effectiveness of water treatment methods and antimicrobial resistance patterns of isolates of *Salmonella* serotypes. Although efforts have been made to make people practice recognized standard operating procedures and processing standards like Hazard Analysis Critical Control Points (HACCP) and Safe Quality Food (SQF) (Press release, 2006), the regulations are rarely observed. The Sub-County also lack bulk food storage facilities as of now. Majority of the food selling shops in Nakuru North don't have deep freezers for storage and the shops that sell foods are of the open type.

Little is known about the state of salmonellosis in Nakuru North Sub-County despite the observed cases of salmonellosis in the area (ROSA, 2007). Although epidemiological studies show 48.1 % cases of salmonellosis (Hlupheka, 2001) in Nakuru North Sub-County, there is still little information on the current state of affairs. The emergence of multiple-antibiotic resistant *Salmonella*, in another major problem in Nakuru North Sub-County (Okeke *et al.*, 2005).

1.3 Research questions

- i) What are the factors that predispose people to salmonellosis in Nakuru North Sub-County?
- ii) What is the *Salmonella* load of milk, intestinal wastes, fruits and vegetable salads, waste water and different water sources sampled in the area?
- iii) What is the effectiveness of the methods of water treatment in controlling salmonellosis?
- iv) Are the *Salmonella* serotypes isolated from the chosen samples sensitive to antibiotics?

1.4 Study hypotheses

- i) There is no significant relationship between the predisposing factors of salmonellosis and infection of salmonellosis.
- ii) There is no significant relationship between the methods of water treatment and control of salmonellosis.

1.5 Objectives of the study

1.5.1 General objective

To identify the factors that predispose people to salmonellosis, isolate *Salmonella*, carry out sensitivity to antibiotics and assess the effectiveness of water treatment methods in the control of salmonellosis in Nakuru North Sub-County.

1.5.2 Specific objectives

- i) To identify the factors that predispose people in Nakuru North Sub-County to salmonellosis.
- ii) To determine the bacterial load of samples collected from milk, intestinal wastes, fruits and vegetable salads, different water sources and waste water.
- iii) To determine the effectiveness of the water treatment methods in the control of salmonellosis.
- iv) To determine antimicrobial resistance patterns of *Salmonella* serotypes isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global overview of salmonellosis

In majority of developing countries, the incidence of human *Salmonella* infection has continued to increase over the years. *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis have been implicated in causing most of these infections (Buck *et al.*, 2003; Chiu *et al.*, 2004; Sadeyen *et al.*, 2004). By 2010, the cost linked to foodborne infections ranged between 551 million and 2.7 billion Euros in Europe, where *Salmonella* infections was estimated to cost 161 000 Euros (Newell *et al.*, 2010). It is reported that the rate of salmonellosis in the United States is between 15 to 20 cases per 100 000 people annually. The Enter-net surveillance program reported *Salmonella enterica* serovar Enteritidis and Typhimurium as the most predominant organisms identified by the participating countries comprising of over 81 % of all isolates during the period of 2005-2011 (Eurosurveillance, 2011).

Salmonellosis is endemic to rural and urban Sub-Saharan Africa (Srikantiah *et al.*, 2011). In rural Mozambique, the incidence of salmonellosis is 120 cases per 100,000 people annually (Crump and Mintz, 2010). The true incidence of salmonellosis is likely to be 2–3 times this figure, because the incidence of bacteremia among patients who die before reaching the district hospital has not been ascertained in either study (Cox and Pavic, 2010). In Uganda, occurrence of salmonellosis 500 cases per 100,000 people per year (Gomez *et al.*, 2011). In rural Kenya, the estimated minimum incidence of salmonellosis is 88 cases per 100,000 people per year (Kariuki *et al.*, 2012).

With the increasing population in Nakuru North Sub-County, there is an increasing demand for food and water which will force the present resource driven system of these products to a demand driven system which will increase the *Salmonella* transmission (Zessin *et al.*, 2006). Limited information regarding *Salmonella* in milk, sheep and cattle offal, salads, waste water and different water sources in Kenya is currently available which will assist in curbing the problem of salmonellosis.

2.2 Isolation of *Salmonella*

2.2.1 Raw cow milk

Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of different products (Nusrat *et al.*, 2012). It contains most of the nutrients such as carbohydrates, proteins, fats, vitamins and minerals required for the growth of microorganisms, combined with higher water activity. This makes it an ideal medium for bacterial growth and therefore it can be considered one of the most perishable agricultural products because it can also be very easily contaminated (Haeschen, 2000). Raw milk contains microorganisms that are capable of causing diseases such as salmonellosis (Headrick *et al.*, 2001). Because of contamination of milk production systems, it is difficult to avoid contamination of milk with micro-organisms. Therefore determination of microorganisms in milk is a major feature in determining its quality (Rogelj, 2003).

2.2.2 Animal intestinal wastes

Ruminants, such as cattle and sheep, are natural reservoirs for *Salmonella*. *Salmonella* is mostly present within the rumen of these animals and is shed through fecal matter, hence ending up in water sources and farms. Previous studies by Reicks *et al.* (2001) (73 %) and Kunze *et al.* (2007) (30.3 %) have reported differing proportions of *Salmonella* in intestinal samples. Cross contamination can begin during transport with other animals via direct body contact or indirectly via contact surfaces (McEvoy, 2004). Contaminants can be transferred to the digestive system during slaughter by initial cuts with knife and when the animal is feeding (Reid *et al.*, 2000).

These contaminations have the risk of contaminating the entire production chain of the slaughter house. Several factors influence animal intestine contamination. These factors include but are not limited to transport conditions and duration, herd lot mingling, drinking water systems, hide cleanliness, and feed withdrawal, automated hide puller/debunching, extent/efficiency of abattoir cleaning and operative hygiene practices (McEvoy, 2004). Various *Salmonella* strains have been isolated from different animals. However, sheep and cattle are more prevalent carriers than nonruminant animals (Hussein, 2006). Due to the innate presence of *Salmonella* in the intestines, cattle hides have been identified as a central source of microbial contamination when animals lick one another or themselves (Hussein, 2006) especially on rainy season compared to dry season. In another study that investigated the seasonal prevalence of non-typhoidal *Salmonella*, it was established that the bacteria are more prevalent during the rainy season than in the dry season (Pidcock, 2001).

2.2.3 Fruits and vegetable salads

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well being. Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006). Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Carmo *et al.*, 2004). Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor *et al.*, 2009).

Fresh produce such as fruit and vegetable salads are often consumed raw, putting consumers at risk of infection by contaminating organisms such as *Salmonella*. The fresh produce industry in many countries has responded to this by adopting various risk management practices designed to reduce the likelihood of contamination (Ofor *et al.*, 2009). However, despite this, the number of reported illnesses linked to contaminated produce has increased (Sivapalasingam *et al.*, 2004). Changes in agricultural processing and distribution practices that have enhanced both the supply and range of products such as triple-washing pre-packaged leafy greens using the same water may also have increased the risk for more widespread outbreaks (Greene *et al.*, 2008).

Salmonella is the most commonly reported bacterial pathogen, accounting for nearly half of the outbreaks due to bacteria (Sivapalasingam *et al.*, 2004). A wide spectrum of produce vehicles have been associated with *Salmonella* infections. Several large-scale outbreaks have been linked to consumption of tomatoes (Cummings *et al.*, 2001; Gupta *et al.*, 2007; Greene *et al.*, 2008) and melons (Bowen *et al.*, 2006; Munnoch *et al.*, 2009). In 2008, jalapeño and serrano peppers were vehicles for a large multistate outbreak of *Salmonella* serovar Saintpaul infections (CDC, 2008). Examples of other outbreaks of *Salmonella enterica* linked to ready-to-eat plant produce include an outbreak in Scandinavia and the UK of serovar Thompson infections associated with consumption of rocket leaves (Nygard *et al.*, 2008), an outbreak of serovar Anatum infections in Denmark linked to imported basil (Pakalniskiene *et al.*, 2009) and an outbreak of serovar Senftenberg infection associated with imported Israeli basil affecting most countries in Sub-Saharan Africa (Pezzoli *et al.*, 2008).

Thus despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased in recent years (Beuchat, 2002). Enteric pathogens such as *Salmonella* are among the greatest concerns during food-related outbreaks (Buck *et al.*, 2003). Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage (Beuchat, 2002). These increases in fruits and vegetables-borne infections may have resulted from increased consumption of contaminated fruits and vegetables outside the home as most people spend long hours outside the home. In Kenya for instance, street vending of handy

ready-to-eat sliced fruit and vegetables has recently become very common and the market is thriving (Muinde and Kuria, 2005). All of the above mentioned studies however failed to quantify the pathogen with reference to the storage pH and time so as to compare their values with the World Health Organization recommended values in fruits and vegetables.

2.2.4 Waste water

Waste water play the main role in the transmission of *Salmonella*. It's estimated that 2.4 billion people have no access to right infrastructure for safe disposal of waste water (Smith, 2002). Thus in many parts of the world, particularly Africa, Latin America, Caribbean and Asia, a greater percentage of waste water gets discharged to the environment without treatment (WHO, 2000). Surface water is often contaminated by urban wastewater, by effluents of meat industries and by wastewater from livestock ranches, involving different serotypes of *Salmonella* (Rusin *et al.*, 2000). The serotypes isolated from human samples do not always coincide with the serotypes isolated from wastewater. In Spain, *Salmonella enterica* serovar Enteritidis (50.7 %), *S. enterica* serovar Typhimurium (23.2 %), and *S. enterica* serovar Hadar (4.7 %) were the most frequent serotypes isolated from clinical human samples in the year 2000 while *S. enterica* serovar Anatum was the one most often isolated from water samples (Usera *et al.*, 2001).

Although wastewater is treated to eliminate pathogenic microorganisms and reduce waterborne transmission, numerous studies indicate that conventional waste water treatment does not guarantee their complete elimination given differences in pH and

temperature of the waste water sample under study (Ngari *et al.*, 2011). According to Howard *et al.* (2004), treated waste water contains MPN of 45/100 ml *Salmonella*. The survival of *Salmonella* despite treatment implies the possibility of selection of the most resistant strains, or the acquisition of resistance through the transference of genetic material. A further study was done by Amoah *et al.* (2005) involving the association of salmonellosis occurrences and waste water. Their study indicated a significant association between the two. With all these studies in place none quantified *Salmonella* pathogens in waste water in Nakuru North Sub-County.

2.2.5 Water sources in Nakuru North Sub- County

In Kenya, salmonellosis is among the major illnesses affecting people living in urban and peri-urban areas. Nakuru North Sub-County has several water sources which include ground water, community taps and river water (APHRC, 2002). Where ground water is used as a source of domestic water, use of pit latrines is not recommended because the two are incompatible unless the water table is extremely low and soil characteristics are not likely to contribute to contamination of ground water. Where ground water and pit latrines coexist, it is difficult to give a general rule for all soil conditions. However, the commonly used guideline is that the well should be located in an area higher than and at least 15 m from the pit latrines and should be at least 2 m above the water table. Available evidence shows that increased lateral separation between the source of pollution and groundwater supply reduces the risk of faecal pollution (ARGOSS, 2001).

Pollution of river waters with *Salmonella* is on a steady increase in the recent past (Niyogi, 2005; Abraham, 2010). The major source of microbes in river water is faeces from human and other mammals. Entry of pathogens into rivers can occur either from a point source, non- point sources or both. Non-point source microbial pollution of rivers occurs from rainwater surface run-offs, storm sewer spillages or overflow, while point-source pollution comes from discharge of untreated or partially treated effluents from wastewater treatment plants (Petersen *et al.*, 2005; Donovan *et al.*, 2008). The impact of river pollution on human health depends mainly on the water uses, as well as the concentration of *Salmonella* in the water (Niyogi, 2005).

Water from community taps is a reliable source of water supply, because it is often unpolluted due to restricted movement of pollutants in the soil profile. However, they are most susceptible to contamination when cracks develop on them leading to contamination with *Salmonella* (Nassinyama *et al.*, 2000; Adejuwon, 2011). Thus, contamination of drinking water from community taps is of primary importance due to the danger and risk of water related diseases (WHO, 2011).

2.3 Methods of water treatment

The most important way to obtain safe drinking water for a community is to isolate and protect its source from contamination with faecal matter, household garbage, industrial waste, mining, quarrying activities, agricultural runoffs of fertilizers, herbicides and pesticides (Gadgil, 2000). Pollution and contamination occur in many places where the water source cannot be protected, and therefore, such water requires treatment before

consumption. Treating contaminated water at the source is a difficult and expensive endeavor, but institutions and national governments have traditionally focused on implementation of large, centralized treatment systems (Montgomery and Elimelech, 2007).

These types of systems generally serve only urban areas, due to their population density, high capital costs, lack of proper operation and over-reliance on technology that is not affordable or well-maintained (Montgomery and Elimelech, 2007). Moreover, previous studies have shown that the water point of use (POU) treatment is more effective in preventing *Salmonella* compared to treatment at the source point (Clasen *et al.*, 2001).

2.3.1 Boiling of water

Boiling is considered the world's oldest, most common, and one of the most effective methods for treating water. Boiling kills all microorganisms in water when the temperature reaches 100 degrees Celsius (Clasen, 2007). For this method of water treatment to be effective, it is recommended that water should be allowed to boil for up to a minimum of 1 minute (Okoko *et al.*, 2012). For every 1000 feet above sea level, add one minute of boiling to the initial 1 minute. If the water pot is covered, it will shorten the time to reach boiling point (CDC, 2009). This method is recognized as the safest treatment. Water is heated over a fire or stove until it boils. Different fuels such as wood, charcoal, biomass, biogas, kerosene, propane, solar panels, and electricity can be used depending on local availability and cost (Latagne *et al.*, 2008). Though boiling of water

significantly improves the microbiological quality of drinking water, boiled and stored drinking water is not always free of *Salmonella* contaminations (CDC, 2009).

2.3.2 Chlorination

Chlorine is the most widely used chemical for disinfecting water because it is affordable (Sobsey, 2002). In disinfection, gaseous chlorine (Cl_2) or liquid sodium hypochlorite bleach, (NaOCl) is added to, and reacts with water to form hypochlorous acid. In the presence of bromine, hypobromous acid is formed. Both chlorine and bromine are in the “halogen” group of elements, and have similar chemical characteristics. Hypochlorous and hypobromous acid form strong oxidizing agents in water and react with a wide variety of compounds, which is why they are such effective disinfectants (Richardson, 2002).

The most practical form of free chlorine for point-of-use water disinfection is liquid sodium hypochlorite (Sobsey, 2002); occasionally, calcium hypochlorite is also used (Arnold and Colford, 2007). It is recommended that the chlorine solution be produced by a local manufacturer or in the community itself using water, salt and an electrolytic cell (CDC, 2001). Both types of hypochlorite solution are inexpensive, and have the additional advantage of providing residual protection against recontamination for hours to days (Sobsey, 2000). In a study carried out by Kirchoff *et al.* (2000), utilizing a double-blind methodology, no differences were found in community cases of *Salmonella* outbreak following chlorination.

There are several drawbacks to the use of chlorine, in addition to its ineffectiveness against chlorine-resistant pathogens. If large quantities of organic material are present, they may lead to ineffectiveness against *Salmonella* since chlorine binds to the organic material, thereby blocking water disinfection (Crump *et al.*, 2007). The addition of large quantities of chemical may also produce an unpleasant taste and odor, reducing willingness of people to drink the water (Kirchoff *et al.*, 2000). In addition, chlorine disinfection by-products (DBPs) have been shown to pose a substantial risk to human health, including an increased risk of cancer of the bladder and of the lower intestinal tracts (Leavens *et al.*, 2007). Despite the accrued risk of bladder cancer with this method of water treatment, chlorination still remains the most commonly used method in Nakuru North Sub-County (ROSA, 2007).

2.3.3 Filtration

Among conventional water filters, the most promising innovations are ceramic water filters, which are manufactured in a basic pot shape or in a tube shape, often called a candle-filter (Makutsa *et al.*, 2001). Pathogens are removed from the contaminated water as it passes through the filter from the top compartment to the lower storage compartment. Many filters are impregnated with colloidal silver or silver nitrate as a bacteriostatic agent which may aid in the overall reduction of pathogens (Nzung'a *et al.*, 2013).

In locally made ceramic filters, factors such as design, method of production and quality assurance vary widely (Brown, 2007). Studies have shown that the majority of bacteria

are removed mechanically through the pores, but a silver coating is necessary to achieve 100 % reduction in *Salmonella* (Latagne *et al.*, 2008). Laboratory tests have shown an effective 100 % separation of *E. coli* from water, indicating that filtration is effective in removal of pathogens from water (Yang *et al.*, 2007). However, porous hydroxyapatite (HA) has not yet been tested thoroughly in combination with silver coatings, or examined as a viable alternative to other ceramics (Wegelin *et al.*, 2005). Currently, filtration is an increasingly marketed treatment method that is being adopted by thousands of households in Nakuru North through the advocacy of non-governmental organisations like Lifestraw (Cleary, 2012). However, this is not yet widely used (Hutton *et al.* 2007; CDC, 2008).

2.3.4 Solar disinfection

Electromagnetic radiation emitted from the sun can be harnessed for point of use (POU) water disinfection (Mintz *et al.*, 2001). A renewed interest in solar disinfection has led to the development of the SODIS system (Weglin, 2001). Inactivation of pathogens is achieved through the destructive effects of UV radiation (“optical disinfection”), through increased temperature (solar pasteurization or solar distillation), or through the synergistic effects of temperature and optical mechanisms (Mintz *et al.*, 2001). However, the exact mechanism by which SODIS inactivates pathogens is not yet fully understood (Mani *et al.*, 2006).

Several studies have examined the efficacy of SODIS (under simulated or laboratory conditions) for inactivating particular strains of pathogens (Sommer *et al.*, 2001). A

serious concern with the SODIS system is the possibility of a phenomenon known as photorepair (Sarah, 2010). This occurs when the enzyme photolyase is activated by exposure to wavelengths of 350-450 nm and begins to repair damage that has been done to the cell (Bohrerova and Linden, 2007). In a photorepair experiment, excess exposure of water to sunlight leads to the bacteria DNA being unreversibly damaged leading to negative results (Onyeka and Ochieng, 2009).

Another related concern with the use of SODIS is the possibility of dark-repair mechanisms, such as those induced by the *recA* gene and subsequent RecA protein produced by the gene. The RecA protein coordinates DNA repair, cell division, and a number of other cellular processes following UV radiation (Stohl *et al.*, 2003). The dark repair mechanism is important if water is irradiated and then stored in a dark place. Following UV fluences (doses) higher than 400 J/m², the German standard for drinking water disinfection, researchers found indication of *recA* gene activity (*recA* mRNA) in opportunistic bacteria using Northern blot analysis (Jungfer *et al.*, 2007). Whereas laboratory studies have had exceptional success using the SODIS system, with little to no pathogenic contamination remaining after several hours of sunlight exposure, limited information is available on effectiveness of SODIS in the field (Rose, 2006).

2.4 Antimicrobial resistance

Indiscriminate use of antimicrobial drugs in animals and humans is the major cause of drug resistance today (Bogaard and Stobberingh, 2000; Threlfall *et al.*, 2000). A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to

animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (White *et al.*, 2001).

In recent years, the emergence and global dissemination of multi-drug resistant typhoidal strains has posed major public health problems in the developing countries, and over the past decade it is assuming epidemic proportions (Okeke *et al.*, 2005). Drug resistant levels have raised major concerns especially in ceftriaxone and ciprofloxacin antibiotics. These antibiotics are often commonly prescribed (Kariuki *et al.*, 2004).

Therefore the emergence of *Salmonella* strains that are resistant to commonly used antimicrobials should be particularly noted by clinicians and microbiologists for better control. This control is most efficiently achieved through the reduction of antimicrobial use (Madhulika *et al.*, 2004). Prudent usage in animal feeds should be combined with good husbandry, abattoir practice, and hygiene at all stages in the food production chain, from processing plants to kitchens and food service establishments. Although some countries have succeeded in reducing the frequency of *Salmonella* in poultry, it is unlikely that the eradication of *Salmonella* in domestic animals could be possible in the foreseeable future (White *et al.*, 2001). The increased occurrence of drug-resistant pathogens in food of animal origin emphasizes the general need for thorough cooking prior to consumption (Walia, 2006). Education of food handlers is an essential step towards reducing the incidence of foodborne diseases resulting from cross-contamination during processing and preparation of foods (WHO, 2005).

2.5 Global trends in resistance pattern

In the recent past, there has been witnessed expansion of resources and the technological advances which have availed large quantities of drugs in developing countries than ever before (Nierop *et al.*, 2005). This has lead to a large population having an access to improved medical care compared to there before. However, the availability and use of these drugs promote development of drug resistance (Kimang'a, 2012).

In Kenya more than 50 % of non-typhoidal *Salmonella* isolate are multi-drug resistant (Okeke *et al.*, 2005). In Ethiopia, resistance pattern of *Salmonella* isolates from chickens indicated a large proportion (60 %) of strains resistant to a variety of drugs (Molla *et al.*, 2003). Previous studies in Ethiopia, Zimbabwe, India, Spain, Canada, Nepal and United States of America have reported the same trend in resistance (Molla *et al.*, 2003; Carraminana *et al.*, 2004; Suresh *et al.*, 2006; Tankhiwale *et al.*, 2003; Achla *et al.*, 2005).

Increased antibiotic resistance among *Salmonella* has been observed in both the number of isolates and resistance to new antibiotics (Fluit, 2005). In a study in carried out in Nepal, 35 multi-drug-resistant strains out of 132 strains of *Salmonella enterica* serovar Typhi were reported to be showing simultaneous resistance to ampicillin, chloramphenicol, and cotrimoxazole (Marthi, 2003). Although there were no isolates resistant to ciprofloxacin, 69.23 % of 52 isolates tested for minimum inhibitory concentration of ciprofloxacin showed reduced susceptibility and 76 % of 112 strains tested for nalidixic acid were resistant (Khanal *et al.*, 2007). There are reports of

Salmonella resistant strains isolated from The Netherlands (Duijkeren *et al.*, 2006), France (Weill *et al.*, 2006), Portugal (Antunes *et al.*, 2003) and many other countries (Bennasar *et al.*, 2000; Chiu *et al.*, 2004; Alphons *et al.*, 2005).

Between the year 1999 and 2004, several studies reported a drastic increase in *Salmonella* resistance to β -lactams antibiotics (Mohanty *et al.*, 2006; Pokharel *et al.*, 2006; Nayak *et al.*, 2004; Zhao *et al.*, 2007; Frye and Fedorka, 2007). In 2004, *Salmonella* resistant to extended spectrum cephalosporins were identified in 43 countries (Arlet *et al.*, 2006). In a retrospective study in Korea, the resistance rate against chloramphenicol showed mild increase, but the ampicillin, trimethoprim/sulfamethoxazole, kanamycin or nalidixic acid remained at a similar level for over 9 years (Yoo *et al.*, 2004). Majority of human cases of non-typhoidal salmonellosis are acquired through the consumption of contaminated food and water. Data on the proportions of serotypes and their resistance patterns in different countries are important for global public health management, because food consumption practices vary in different countries (Onyango *et al.*, 2008). In addition, increasing global travel and food trade increase the likelihood of acquiring infections from non-domestic sources (Lauderdale *et al.*, 2006).

In a recent study carried out in Nairobi, Kenya, involving isolation of *Salmonella* and *Vibrio* from abattoirs sewage, *Salmonella* isolates were most resistant to lincomycin, ampicillin and methicillin and most sensitive to chloramphenicol, gentamicin and cotrimoxazole. The study also demonstrated that the isolates were resistant to antibiotics commonly used as feed additives (tetracycline, streptomycin and sulfonamides) or

therapeutics (penicillin and tetracycline) (Atieno *et al.*, 2013). The high resistances observed in this study were attributed to presence of capsular K and Vi antigens in *Salmonella* which protect them from access to antimicrobials (Miranda *et al.*, 2002).

This study was aimed at determining the *Salmonella* serotypes circulating in Nakuru North Sub-county and their responses to the currently used antibiotics in treating salmonellosis. Currently, emphases have been put to preventive rather than curative treatment of salmonellosis (CDC, 2009). This creates a need for the study of the control methods of salmonellosis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Nakuru North Sub-County in Nakuru County. The Sub-County is located 160 km Northwest of Nairobi at an altitude of 1,859 m above sea level, with an area of 593 km² (Fig 3.1). It has a population density of 25.3 per km². It is specifically located between longitudes 35° 28' and 35° 36' East and latitude 0° 13' and 1° 10' South. The main livelihoods in the Sub-County are mixed farming, employment and trade (KFSSG, 2008). It was carried out during the period of May to September 2012. The isolation of *Salmonella* was carried out at Rift Valley Regional Water Testing Laboratories while biochemical, serotyping and antimicrobial resistance was carried out at Amec laboratories, Nakuru.

3.2 Sample size

With the confidence interval of 95 % and maximum allowable error of 5 %, the sample size was determined using the following formula by Fisher (Fisher, 1998);

$$n = \frac{Z^2 pqD}{d^2}$$

Where; n = sample size, p = anticipated prevalence which was 3 % (0.03) in this study, q = failure which was calculated as 100-3 giving 97 % (0.97), Z = is the appropriate value from the normal distribution for the desired confidence level which was 1.96 in this study, d = allowable error (0.05) and D = design effect which was given a value of 2 because replication was carried out. Based on 3 % prevalence and Z value of 1.96 the sample size was;

$$n = \frac{1.96^2(0.03 \times 0.97)}{(0.05^2)} = 90$$

3.3 Identification of predisposing factors to salmonellosis

A sample of 90 respondents from the study population was taken at random. All the people in the study area were assigned numbers from which the computer was used in picking the sample. They were requested to complete a questionnaire. The risks were calculated and rated among the respondents.

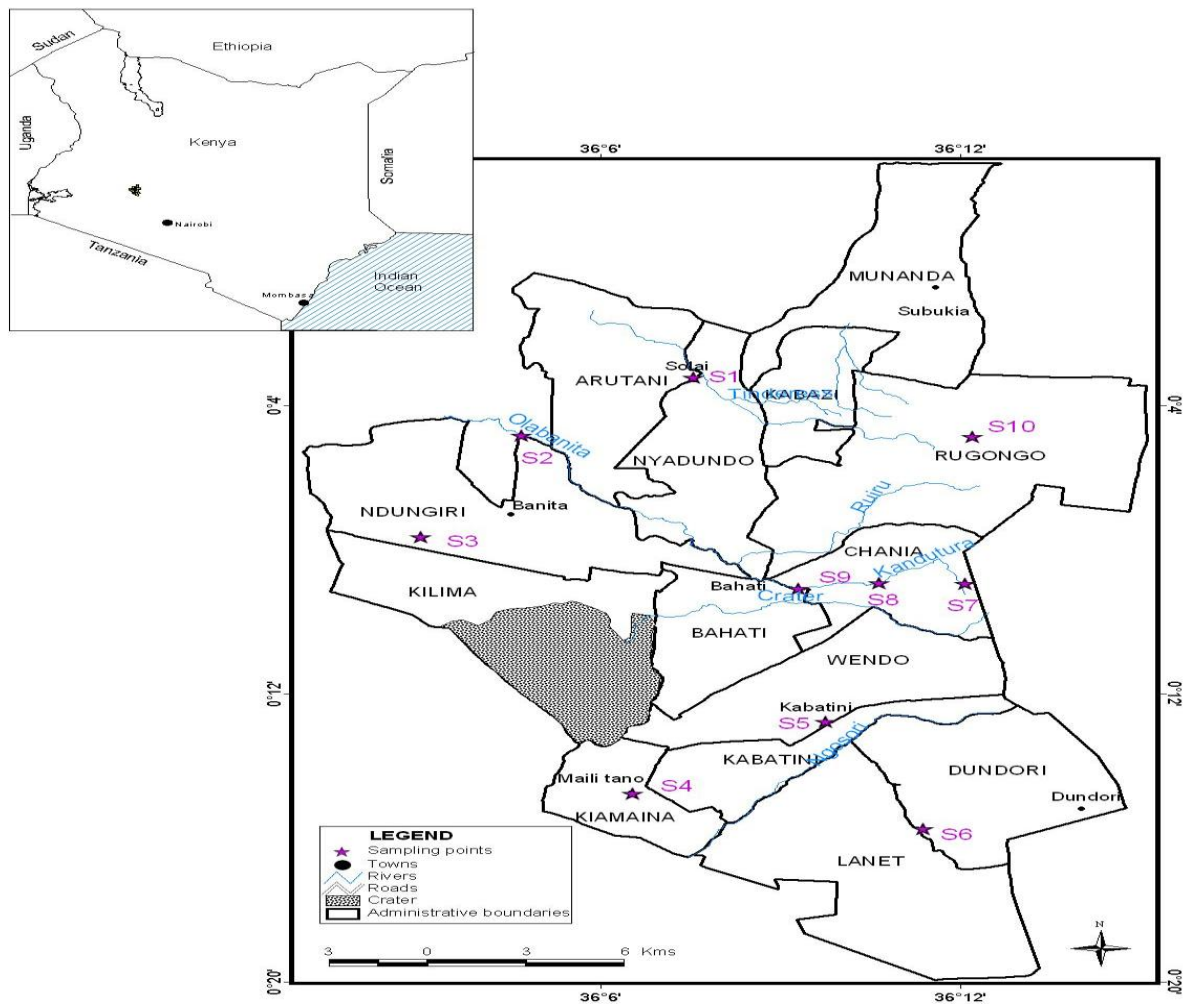


Figure 3.1: Map of Nakuru North Sub-County (KFSSG, 2008).

3.4 Sample collection

A total of 630 samples were collected from vegetables and fruits salads, raw milk from milk kiosks, intestinal wastes from butcheries, waste water samples from the surrounding environment, water from community taps and water from River Kandutura and treated water through boiling, chlorination and filtration from the households and through solar disinfection from the experiment that was carried out. Samples of water from River Kandutura were collected from three regions; the upstream, midstream and downstream which were 15 km apart. All samples were collected using sterile glass specimen bottles. The bottles were sterilized using sodium hypochlorite followed by thorough rinsing using dionized water. The temperature of the samples at the point of collection was noted in each sample before being transported to Rift Valley Regional Water Testing Laboratories in Nakuru town. They were stored at a temperature of 4 °C until processing.

3.5 *Salmonella* isolation procedure

The study was conducted using the conventional methods for the detection of *Salmonella*. Briefly, 25 g of fruit and vegetable salads and intestinal wastes were taken and each category was put in a sterile stomacher bag and 225 ml of buffered peptone water (Sifin, Germany) was added, and homogenized using a laboratory blender (Stomacher 400, Seward, England) for 2 min. This was followed by transferring aseptically 1 ml and 0.1 ml aliquot of the samples into 10 ml of selenite F broth for 24 h at 37 °C (Baumler *et al.*, 2000). Following incubation, a loopful of each culture was streaked onto one plate of xylose lysine desoxycholate and another of *Salmonella-Shigella* agar (Sifin, Germany) medium and incubated at 37 °C for 24 h.

Hundred milliliters of each of the samples of milk, waste water, water from water sources and treated water, was filtered using membrane filters (Whatman GmbH, Germany), pore size 0.45 μm , 47 mm diameter (Plate 3.1). The filters were placed in peptone water for 2 min before being transferred to selenite F broth for selective enrichment. After incubation at 37 °C for 24 h, the filters were placed aseptically on XLD and *Salmonella-Shigella* agar and incubated at 37 °C for 24 h. The plates (XLD and S-S) were examined for the presence of *Salmonella* colonies. Organisms having transparent colonies with black centres on S-S agar and reddish colonies possessing black centres on XLD agar were suspected to be *Salmonella*. These isolates were counted using colony counter technique before being subjected to further confirmation by API 20E and serotyping after enumeration using colony counter technique (ISO 6579, 2002).



Plate 3.1: Membrane filtration of liquid samples.

3. 6 Analytical Profile Index (API 20E)

Biochemical identification was carried out using API 20E strip kit (bioMérieux®, Inc., France). The reagents used included API NaCl 0.85 % medium, API 20 E reagent kit, Zn reagent, oxidase, mineral oil and API 20E Analytical Profile Index. The strips were prepared by the use of an incubation box (tray and lid). In this preparation, 5 ml of distilled water was distributed into the honey-combed wells of the tray to create humid atmosphere. Inocula of pure isolates were, emulsified in 5 ml of normal saline 0.85 % NaCl to achieve a homogeneous bacterial suspension. Anaerobiosis in the tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), H₂S production (H₂S), Urease (URE), citrate utilization (CIT), fermentation/oxidation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhaminose (RHA), melibiose (MEL) and arabinose (ARA), gelatinase (GEL), tryptophane deaminase (TDA), fermentation/oxidation of sugars sucrose (SAC) and amygdaline (AMY), Indol (IND) and acetoin (VP) production was maintained by overlaying with mineral oil. The incubation box was closed and incubated at a temperature 37 °C for 24 h as described by the manufacturer and the results were determined according to API 20E catalogue (Ohud *et al.*, 2012).

3.7 Serological test

Pure *Salmonella* isolates were inoculated on nutrient agar plates overnight and then typed against their respective antisera for 'O', 'Vi' and 'H' agglutination reactions (Bopp *et al.*, 2003). Selected strains were subjected to slide agglutination with polyvalent antisera (Behring, Marburgy, Germany). Briefly, a loopful of bacterial mass from suspected

colonies grown on nutrient agar was mixed with a drop of antiserum on a carefully cleaned glass slide so as to get a homogenous and turbid suspension. Positive results were recorded if agglutination occurred within 20 min after shaking against dark background. In order to exclude any spontaneous agglutination (auto agglutination) a negative control using physiological saline solution and bacterial colony to be tested was included in the test.

3.8 Solar disinfection

An experimental design adopted from Oates *et al.* (2003) was used in order to investigate the effectiveness of the method in the study area. Three sets labeled A, B and C each having 6 bottles were used. Set A had infected water from the community taps and River Kandutura, B had water inoculated with commercial *Salmonella* while C was a control with contaminated water from community taps and river Kandutura. Set A and B were exposed to sunlight for 5 hrs while set C was kept in the dark. *Salmonella* analysis was carried out on hourly basis. Exposure to sunlight was carried out for five continuous hours starting at 10 a.m each day for 90 sunny days (Oates *et al.*, 2003).

3.9 Antimicrobial sensitivity testing

The antimicrobial susceptibility testing was carried out by use of Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) (Bauer *et al.*, 1996). Sterile wire loop was used to pick 3 colonies of each *Salmonella* serotype and emulsified in 3 ml of sterile physiological saline. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the

turbidity matched the 0.5 McFarland Standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20 ml of Mueller-Hinton agar in a 150 mm disposable plate (STERLIN, UK).

The inoculated plates were air dried, and antibiotic discs (ABTEK BIOLOGICAL LTD., UK) were placed on the agar using sterile forceps and were gently pressed down to ensure contact. The following 7 antibiotic discs were used; cephalexin (CL, 30 µg), nalidixic acid (NA, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP 5 ug), gentamicin (CN, 10 µg), amoxicillin (AML, 10 µg) and sulfa-trimethoprim (SXT, 25 µg). The plates were incubated aerobically at 37 °C for 24 h. *Escherichia coli* (ATCC 25922) was used as control. The zones of inhibition were measured in millimeters and graded according to sensitive, intermediate or resistant (Barbara *et al.*, 2000).

3.10 Data management and analysis

Chi-square test was used to determine the association between factors that predispose people to salmonellosis and cases of salmonellosis while correlation test was used in calculating association of isolated *Salmonella* and the factors under study. Tukey's Honest significance at 5 % level of significance was used to separate the means. Anova was used in determining whether the differences in *Salmonella* isolates were statistically significant between Maili 5, Bahati and Kabatini. The confidence intervals for antimicrobial resistance were estimated using win Episcopes (win Episcopes[®] version 2.0) and Epicalc 2000 (version 1.02) software. The results were analyzed using Statistical Package for Social Sciences (SPSS) version 11.50 software.

CHAPTER FOUR

RESULTS

4.1 Predisposing factors of salmonellosis

4.1.1 Ages of the respondents

In a total of 90 respondents that were randomly sampled; 30 were from Maili 5, 30 from Bahati and 30 from Kabatini. The respondents' were aged between 18-20 (8.9 %), 20-29 (12.2 %), 30-39 (20 %), 40-49 (26.7), 50-59 (20 %) and above 60 (12.2 %) (Table 4. 1).

4.1.2 Education level of the respondents

In a total of 90 respondents, 44 (48.9 %) of the respondents had attained primary level of education, 35 (38.9 %) had attained secondary level of education and 11 (12.2 %) University or college (Table 4.1). Level of education was found to be a risk for transmission of *Salmonella* ($\chi^2 = 10$, d f = 4, $p < 0.019$) with respondents having low level of education being at a higher risk of infection.

4.1.3 Occupation of the respondents

In this study area, out of 90 respondents, 29 (32.2 %) of the respondents were self employed, 25 (27.8 %) full time employment, 4 (4.4 %) part time employment while 4 (4.4 %) were on sick leave. In addition, 24 (26.8 %) respondents were unemployed, students 2 (2.2 %) while 2 (2.2 %) were on voluntary retirement (Table 4.1). Occupation of respondents influenced the transmission of *Salmonella* ($\chi^2 = 30.7$, d f = 12, $p < 0.024$).

Table 4.1: Participants social-demographic data in Nakuru North Sub-County from May to September 2012

Demographics	Demography clusters	Frequency (n)	Percent (%)	P – value
Age	18-20	8	8.9	
	20-29	11	12.2	
	30-39	18	20	
	40-49	24	26.7	
	50-59	18	20	
	Above 60	11	12.2	
Education level	Primary	44	48.9	0.019
	Secondary	35	38.9	
	University/College	11	12.2	
Occupation	Student	2	2.2	0.024
	Employed full time	25	27.8	
	Employed part time	4	4.4	
	Self employed	29	32.2	
	Sick leave	4	4.4	
	Voluntary retirement	2	2.2	
	Involuntary retirement	-	-	
Unemployed	24	26.8		
Cleaning of utensils	After each use	55	61.1	0.0416
	Much later	35	38.9	
Hand washing	Have knowledge	55	61.1	0.027
	No knowledge	35	38.9	
Human waste disposal	In latrine/toilet	73	81.1	0.044
	In a nearby bush	17	18.9	
Animal waste disposal (n=36)	In a pit	1	2.8	0.029
	Scattered in the compound	1	2.8	
	Used as manure	34	34	
Blocked sewers	None	48	53.4	0.028
	Occasionally	42	46.6	
Waste water disposal	Pouring outside the house	33	36.7	0.022
	In a pit	2	2.2	
	Drainage pipe	55	61.1	
Water source	River	41	45.6	0.03
	Community tap	5	5.6	
	Well	44	48.8	
Water treatment	Treat water	27	30	<0.05
	Do not treat	63	70	
Method of water treatment (n = 27)	Boiling	15	55.6	0.89
	Chlorination	7	25.9	
	Filtration	5	18.5	

Frequency and percent of the respondents having a corresponding attribute.

4.1.4 Sex of the respondents

In a total of 90 respondents, 48 (53.06 %) were females while 42 (46.94 %) were males (Figure 4.1). However, sex of the respondents did not significantly influenced occurrence of salmonellosis ($\chi^2 = 1.8$, d f = 2, $p > 0.05$).

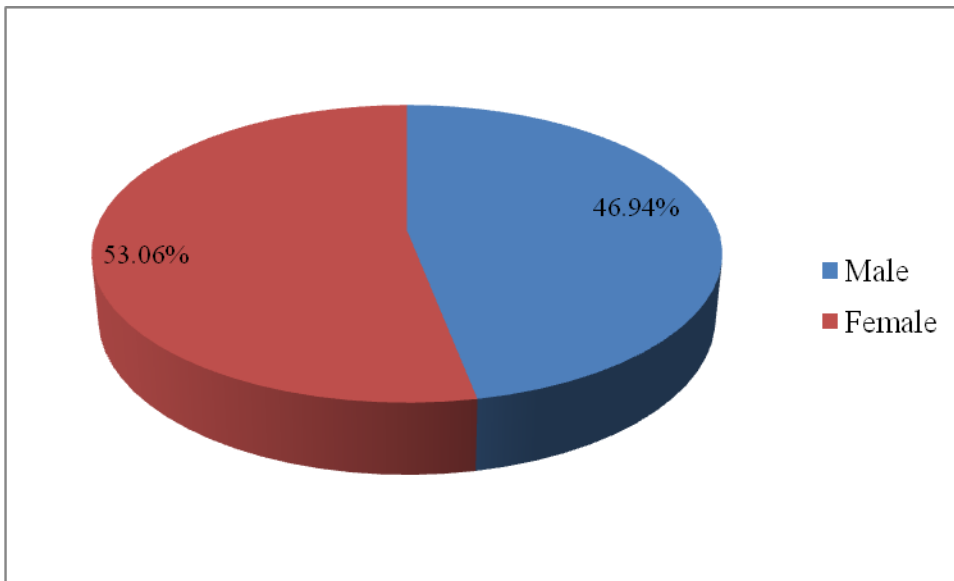


Figure 4.1: Distribution of the respondents according to sex in Nakuru North Sub-County from May to September 2012.

4.1.5 Storage of food

In a total of 90 respondents, 20 (22.5 %) stored their foods at room temperature and covered them while in 70 (77.5 %) respondents food was stored at room temperature but not covered (Figure 4. 2). Food storage methods after preparation influenced emergence of salmonellosis ($\chi^2 = 6.4$, d f = 2, $p < 0.0375$).

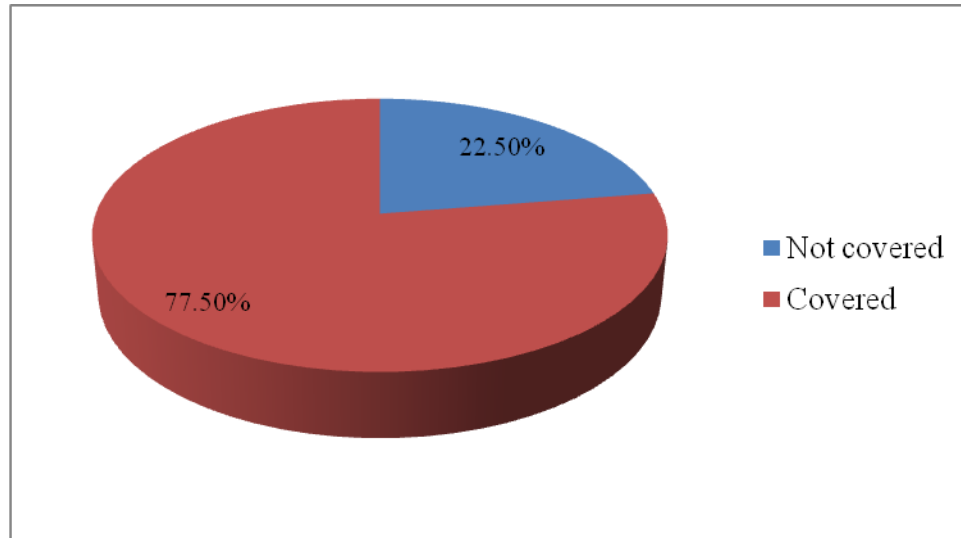


Figure 4.2: Distribution of the respondents according to food storage in Nakuru North Sub-County from May to September 2012.

4.1.6 Cleaning of kitchen utensils

After use of the utensils, 55 (61.1 %) respondents indicated that they cleaned their utensils immediately after use while 35 (38.9 %) respondents did not clean them immediately after use (Table 4.1). Cleaning of kitchen utensils posed a risk for salmonellosis ($\chi^2 = 6.4$, $d f = 2$, $p < 0.0416$).

4.1.7 Hand washing practices

In 55 (61.1 %) out of 90 respondents there was some knowledge on reduction of contamination by washing of hands, while 35 (38.9 %) of the respondents did not have that knowledge (Table 4.1). Hand washing practices were a risk to contraction of salmonellosis ($\chi^2 = 7.2$, $d f = 2$, $p < 0.027$).

4.1.8 Human waste disposal

Human waste disposal among the respondents was also evaluated. In a total of 90 households, 73 (81.1 %) of the respondents dispose off their human waste in toilets or latrines while the rest 17 (18.9 %) respondents at the nearby bush (Table 4.1). Chi-square test indicated that human waste disposal is a factor which influences transmission of *Salmonella* ($\chi^2 = 6$, d f = 2, $p < 0.044$).

4.1.9 Animal waste disposal

In a total of 36 respondents who keep animals, 34 (94.4 %) used their animal wastes as manure in their farms while 1 (2.8 %) respondent threw his animal wastes in pits. In addition, 1 (2.8 %) respondent was found to leave his animal wastes scattered in the compound (Table 4.1). In this study, animal waste disposal was found to significantly predispose people to salmonellosis ($\chi^2 = 10.7$, d f = 4, $p < 0.029$).

4.1.10 Sewerage in the area

According to 42 (46.6 %) respondents, presence of blocked sewers was observed occasionally in the compounds. In 48 (53.40 %) respondents there were no blocked sewers in the compounds (Table 4.1). Presence of blocked sewers was statistically significant to the transmission of salmonellosis ($\chi^2 = 10.3$, d f = 2, $p < 0.028$).

4.1.11 Waste water disposal

In this area, 55 (61.1 %) respondents, waste waters run into drainage pipes while 33 (36.7 %) respondents poured their waste water outside the house at a central place. In addition,

2 (2.2 %) respondents threw their waste water in pits (Table 4.1). Waste water disposal influenced transmission of *Salmonella* ($\chi^2 = 11.5$, d f = 4, $p < 0.022$).

4.1.12 Sources of water for use in the households

Water was obtained from community taps as per the reports from 44 (48.8%) of the respondents, 41 (45.6 %) from the rivers while only 5 (5.6 %) of the respondents got their water from wells. River and tap water are risk factors to transmission of *Salmonella*. However, well water did not influence significantly the transmission of *Salmonella* (Table 4.1).

4.1.13 Water treatment in the households

In a total of 90 respondents, 27 (30 %) respondents treated their drinking water while the rest 63 (70 %) use untreated water (Table 4.1). Water treatment significantly influenced occurrence of salmonellosis ($\chi^2 = 5.7$, d f = 2, $p < 0.05$).

4.1.14 Methods of water treatment

In a total of 27 respondents that treated their drinking water, 15 (55.6 %) respondents boiled their waters, 7 (25.9 %) chlorinated theirs while 5 (18.5 %) of the households filtered their water (Table 4.1). The method of water treatment did not significantly influence the transmission of salmonellosis ($\chi^2 = 2.3$, d f = 4, $p > 0.05$).

4.2 Isolation of *Salmonella*

Salmonella was isolated from raw milk, animal intestinal wastes, raw fruits and vegetable salads, waste water, different water sources and treated water (Plate 4.1).

4.2.1 *Salmonella* load of raw milk

The average *Salmonella* load from Maili 5 was 1.3×10^4 cfu/100 ml which was higher compared to samples from Bahati (4.8×10^3) and Kabatini (4.6×10^3) (Table 4.2). Average pH of milk from Maili 5 and Bahati was 7.3 which was lower compared to samples from Kabatini (7.4). In addition, raw milk from Maili 5 had a mean temperature of 18.1 °C, Bahati (17.8 °C) and Kabatini (16.8 °C). The pH ranged from 7.1 in the three sampled areas to 7.6 in Kabatini. The lowest temperature of milk was 16.7 °C at Kabatini with the highest temperature (18.4 °C) being recorded in Maili 5 (Table 4.2).

There was no significant variation in microbial load of raw milk from Maili 5, Bahati and Kabatini ($F= 1.837$, $P > 0.05$). The result also indicated that there was no significant relationship in number of *Salmonella* colony forming units to temperature ($r = 0.603$, $P = 0.588$) or to milk pH ($r = 0.756$, $P = 0.454$). Positive trend indicating increase in milk temperature and pH resulted in increase in *Salmonella* colony forming units.

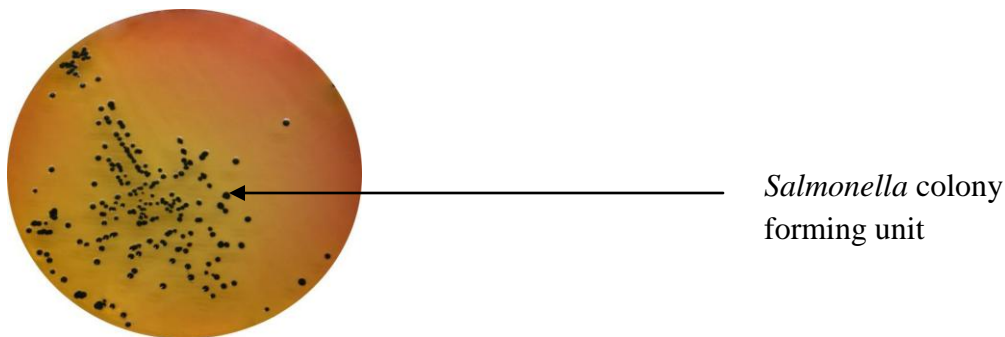


Plate 4.1: *Salmonella* in *Salmonella-Shigella* agar.

Table 4.2: Colony forming units of *Salmonella*, temperature and pH in raw milk samples from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100 ml)			Temperature (°C)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	1.0 x10 ³	1.0x10 ⁴	1.0x10 ⁴	17.6	17.8	16.8	7.1	7.3	7.2
2	2.0x10 ³	1.0 x10 ⁴	1.0x10 ⁴	18.0	17.9	16.7	7.3	7.2	7.4
3	1.0x10 ⁴	1.0x10 ³	1.0 x10 ³	18.4	18.0	16.8	7.5	7.1	7.1
4	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	18.0	17.5	16.8	7.2	7.4	7.5
5	4.0x10 ⁴	2.0 x10 ³	1.0x10 ³	18.4	17.8	16.8	7.4	7.3	7.6
Mean	1.3x10⁴	4.8x10³	4.6x10³	18.1	17.8	16.8	7.3	7.3	7.4

Cfu, colony forming unit.

4. 2.2 *Salmonella* load from animal intestinal wastes

4.2.2.1 *Salmonella* load of sheep intestinal wastes

The mean *Salmonella* load in sheep intestinal wastes from Maili 5 was 1.4×10^4 cfu/100 g which was higher compared to that from Bahati (6.8×10^3 cfu/100 ml) and Kabatini (4.6×10^3 cfu/100 g) (Table 4.3). The temperature in Maili 5, Bahati and Kabatini ranged from 17.4 °C to 18.5 °C observed in Maili 5. The highest pH (7.7) was observed at Kabatini while the least was 7.0 in Maili 5. The mean *Salmonella* obtained from sheep intestinal wastes in the three sampling sites were not statistically significant ($F = 2.018$, $P = 0.19$). There was no significant relationship in either the level of *Salmonella* to temperature ($r = -0.686$, $P = 0.519$) or to pH ($r = -0.854$, $P = 0.348$).

Table 4.3: Colony forming units of *Salmonella*, temperature and pH in sheep intestinal waste samples from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100 g)			Temperature (°C)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	2.0x10 ⁴	2.0x10 ⁴	1.0x10 ⁴	18.0	17.9	17.9	7.3	7.3	7.5
2	1.0x10 ⁴	1.0x10 ⁴	1.0x10 ⁴	18.5	17.8	18.3	7.5	7.6	7.2
3	2.0x10 ⁴	2.0x10 ³	1.0x10 ³	17.6	17.8	17.5	7.4	7.2	7.6
4	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	17.4	18.0	17.8	7.2	7.2	7.4
5	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	17.6	18.2	18.0	7.0	7.5	7.7
Mean	1.4x10⁴	6.8x10³	4.6x10³	17.8	17.9	17.8	7.3	7.4	7.5

Cfu, colony forming unit.

4.2.2.2 *Salmonella* load of cattle intestinal wastes

The mean *Salmonella* load detected from cattle intestinal waste (5.0×10^4 cfu/100 g) in Maili 5 was higher compared to that in Bahati and Kabatini (4.6×10^4 cfu/100 g) (Table 4.4). The temperature ranged between 16.9 °C and 18.9 °C while pH was between 7.1 and 7.6. *Salmonella* isolates from cattle intestinal wastes in the three sampling points were not significantly different ($F = 0.091$, $P = 0.914$). *Salmonella* colony forming units from cattle intestinal wastes were not significantly related to pH ($r = -0.218$, $P = 0.574$) or temperature ($r = -0.250$, $P = 0.517$).

Table 4.4: Colony forming units of *Salmonella*, temperature and pH in cattle intestinal waste samples from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100 g)			Temperature (°C)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	1.0x10 ⁵	1.0x10 ⁴	1.0x10 ⁴	18.4	17.9	17.4	7.4	7.5	7.4
2	2.0x10 ⁴	1.0x10 ⁴	1.0x10 ⁵	18.0	17.9	17.7	7.5	7.3	7.3
3	1.0x10 ⁵	1.0x10 ⁴	1.0x10 ⁵	18.4	17.8	16.9	7.6	7.1	7.1
4	2.0x10 ⁴	2.0x10 ⁵	2.0x10 ⁴	18.9	17.8	17.7	7.2	7.5	7.2
5	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	17.9	18.0	17.8	7.6	7.6	7.5
Mean	5.0x10⁴	4.6x10⁴	4.6x10⁴	18.3	17.9	17.8	7.5	7.4	7.3

Cfu, colony forming unit.

4.2.3 *Salmonella* load of raw fruits and vegetable salads

Salmonella isolated from raw fruits and vegetable salads in Bahati (2.4×10^4 cfu/100 g) was higher compared to that from Maili 5 (1.5×10^4 cfu/100 g) and Kabatini (1.4×10^4 cfu/100 g) (Table 4.5). There was a wide range of storage time of the salads ranging from 5 h in Maili 5 and Bahati to 36 h in Kabatini. The pH in Maili 5, Bahati and Kabatini ranged from 5.5 in Kabatini to 7.6 in Bahati. The mean differences of *Salmonella* in fruits and vegetable salads from Maili 5, Bahati and Kabatini were not statistically significant ($F = 0.614$, $P = 0.562$). In the current study, *Salmonella* colony forming units from raw fruits and vegetables were not significantly related to pH ($r = 0.803$, $P = 0.054$) or to storage time ($r = -0.639$, $P = 0.172$) of raw fruits and vegetable salads.

Table 4.5: Colony forming units of *Salmonella* in raw fruits and vegetable salads, storage time and pH from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100g)			Storage time (Hrs)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	6	24	36	6.0	6.9	5.9
2	1.0x10 ⁴	1.0x10 ⁴	1.0x10 ³	5	10	7	5.7	7.0	5.5
3	2.0x10 ⁴	3.0x10 ⁴	2.0x10 ⁴	12	5	10	6.5	7.1	6.5
4	3.0x10 ⁴	2.0x10 ⁴	6.0x10 ³	12	12	12	7.3	7.4	7.0
5	8.0x10 ⁴	6.0x10 ⁴	4.0x10 ⁴	24	8	9	7.4	7.6	7.4
Mean	1.5x10⁴	2.4x10⁴	1.4x10⁴	11.8	11.8	14.8	6.6	7.2	6.5

Cfu, colony forming unit.

4.2.4 *Salmonella* load of waste water

The mean *Salmonella* load isolated from waste water in Maili 5 was 2.3×10^5 cfu/100 ml, 1.2×10^5 cfu/100 ml in Bahati and 1.4×10^5 cfu/100 ml in Kabatini (Table 4.6). The temperature in Maili 5, Bahati and Kabatini ranged between 17.0 °C and 18.4 °C observed at Bahati. All the samples were alkaline with the least pH being 8.0 in Bahati and Kabatini while the highest was 8.5 in Kabatini. The mean *Salmonella* isolates in the three study sites were however not significantly different ($F = 0.592$, $P = 0.573$). *Salmonella* colony forming units from waste waters were not significantly related to pH ($r = 0.076$, $P = 0.815$) or to temperatures ($r = -0.115$, $P = 0.630$) of waste water.

Table 4.6: Colony forming units of *Salmonella* from waste water, temperature and pH from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100 ml)			Temperature (°C)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	3.0x10 ⁵	3.0x10 ⁵	1.0x10 ³	17.4	18.4	17.8	8.1	8.1	8.1
2	2.0x10 ⁴	2.0x10 ³	3.0x10 ⁵	17.3	18.1	18.4	8.1	8.0	8.1
3	6.0x10 ⁵	2.0x10 ⁵	2.0x10 ⁵	17.9	17.9	18.0	8.1	8.1	8.5
4	2.0x10 ⁵	1.0x10 ⁵	2.0x10 ⁵	18.1	17.0	17.9	8.2	8.0	8.4
5	2.0x10 ⁴	1.0x10 ⁴	2.0x10 ⁴	18.0	17.5	18.2	8.20	8.2	8.3
Mean	2.3x10⁵	1.2x10⁵	1.4x10⁵	17.7	17.8	18.1	8.2	8.1	8.3

Cfu, colony forming unit.

4.2.5 *Salmonella* load of water sources

4.2.5.1 *Salmonella* load of water running in community taps

The *Salmonella* load of water from community taps in Maili 5 was higher (2.4×10^4 cfu/100 ml) compared to Bahati (2.0×10^4 cfu/100 ml) and Kabatini (8.0×10^3 cfu/100 ml) (Table 4.7). The lowest temperature recorded was 17.5 °C and the highest was 18.8 °C both in Kabatini. Water turbidity levels on the other hand ranged from 5.2 NTU in Maili 5 to 8.3 NTU in Bahati while pH ranged from 7.6 in Kabatini to 8.9 in Maili 5. The mean difference in temperature and turbidity in Maili 5, Bahati and Kabatini were not statistically significant ($F = 0.08$ $p > 0.05$). The mean *Salmonella* isolates from the three sampling regions were not statistically significant ($F = 0.75$ $P = 0.499$). *Salmonella* colony forming units from community tap water was not significantly related to temperature ($r = 0.939$, $P = 0.224$) or to pH ($r = 0.132$, $P = 0.916$) and turbidity ($r = -0.008$, $P = 0.995$).

Table 4.7: Colony forming units of *Salmonella*, temperature, turbidity and pH from water running in community taps from Maili 5, Bahati and Kabatini in Nakuru North Sub-County from May to September 2012

Sample No	<i>Salmonella</i> (cfu/100 ml)			Temperature (°C)			Turbidity (NTU)			pH		
	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini	Maili 5	Bahati	Kabatini
1	2.0x10 ⁴	2.0x10 ⁴	1.0x10 ³	18.0	18.5	17.9	5.7	8.2	7.2	8.6	8.1	7.6
2	1.0x10 ⁴	1.0x10 ⁴	3.0x10 ⁴	18.0	17.9	18.8	5.2	8.3	7.3	8.4	7.9	8.2
3	1.0x10 ⁴	1.0x10 ³	1.0x10 ³	18.6	18.2	17.5	5.3	8.2	7.4	8.4	8.0	7.9
4	3.0x10 ⁴	3.0x10 ⁴	1.0x10 ³	18.2	18.1	18.1	7.7	8.1	7.6	8.1	8.2	8.5
5	5.0x10 ⁴	4.0x10 ⁴	1.0x10 ⁴	18.1	17.9	18.5	7.2	8.2	8.0	8.9	7.8	8.0
Mean	2.4x10⁴	2.0x10⁴	8.0x10³	18.2	18.1	18.2	6.2	8.20	7.5	8.5	7.9	8.0

Cfu, colony forming unit; NTU, Nephelometric Turbidity Units.

4.2.5.2 *Salmonella* load of water samples from river Kandutura

Average *Salmonella* load isolated from downstream of river Kandutura was 4.2×10^4 cfu/100 ml and this was higher compared to midstream (1.6×10^4 cfu/100 ml) and upstream (8.2×10^1 cfu/100 ml). The temperature in the upstream, midstream and the downstream ranged between 16.8 °C and 18.9 °C observed in the downstream of the river (Table 4.8). Turbidity levels ranged between 3.2 NTU upstream and 7.5 NTU downstream while pH ranged from 7.1 in the midstream to 8.5 upstream. The mean *Salmonella* isolates from upstream to downstream in this study varied significantly ($F = 15.38$, $P = 0.001$). *Salmonella* colony forming units from river Kandutura's water was not significantly related to temperature ($r = 0.839$, $P = 0.316$) or to pH ($r = 0.122$, $P = 0.916$) and turbidity ($r = -0.008$, $P = 0.995$).

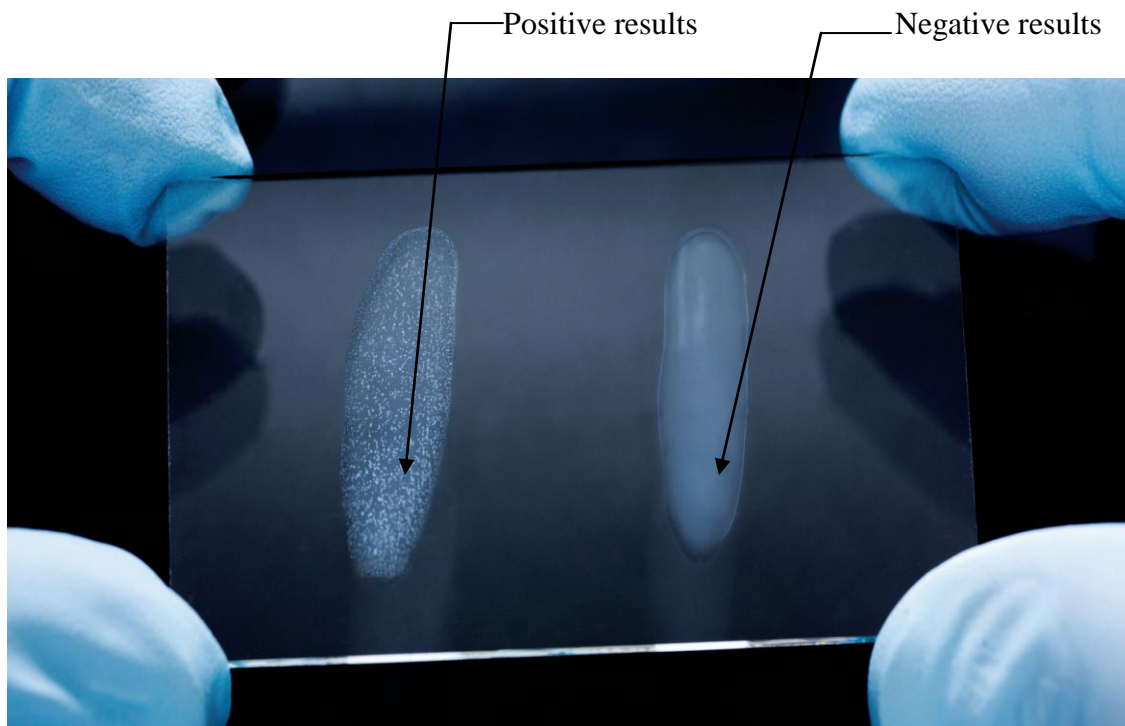


Plate 4.2: Serotyping using slide agglutination test.

Table 4.8: Colony forming units of *Salmonella*, temperature, turbidity and pH in water from river Kandutura in Nakuru North Sub-County from May to September 2012

Sample No.	<i>Salmonella</i> (cfu/100 ml)			Temperature (°c)			Turbidity (NTU)			pH		
	Ustr	Mstr	Dstr	Ustr	Mstr	Dstr	Ustr	Mistr	Dstr	Ustr	Mstr	Dstr
1	1.0x10 ¹	2.0x10 ⁴	3.0x10 ⁴	17.0	17.4	18.0	4.3	5.7	6.9	7.5	8.1	8.2
2	1.0x10 ²	2.0x10 ⁴	6.0x10 ⁴	18.5	18.0	18.9	4.5	6.0	7.2	7.8	7.1	8.1
3	1.0x10 ²	1.0x10 ⁴	4.0x10 ⁴	16.8	19.0	18.4	5.3	5.6	7.2	8.0	8.1	8.1
4	1.0 x10 ²	2.0x10 ⁴	5.0x10 ⁴	18.6	17.1	18.0	5.2	5.1	7.4	8.5	7.2	8.1
5	1.0x10 ²	1.0x10 ⁴	3.0x10 ⁴	17.5	17.0	18.6	3.2	6.0	7.5	7.9	8.1	8.2
Mean	8.2x10¹	1.6x10⁴	4.2x10⁴	17.7	17.7	18.4	4.51	5.68	7.2	7.9	7.7	8.1

Cfu, colony forming unit; NTU, Nephelometric Turbidity Units; Ustr, upstream; Mstr, midstream; Dstr, downstream.

4.3 *Salmonella* load of boiled, chlorinated and filtered water

On average, *Salmonella* isolates from boiled water were highest in Maili 5 (5×10^4 cfu/100 ml) and lowest at Kabatini (1.0×10^4 cfu/100 ml) while from chlorinated water Maili 5 (3.0×10^3 cfu/100 ml) was highest and Bahati (1.0×10^3 cfu/100 ml) was the lowest. In filtered water, Maili 5 had the highest (7.0×10^4 cfu/100 ml) *Salmonella* isolates while Kabatini had the lowest the lowest (3.1×10^4) (Table 4.9).

Table 4.9: Mean *Salmonella* colony forming units in Maili 5, Bahati and Kabatini waters after treatment in Nakuru North Sub-County from May to September 2012

<i>Salmonella</i> (Cfu/100ml)	Maili 5	Bahati	Kabatini	p-Value
Boiled water	5.0×10^4	2.1×10^4	1.0×10^4	0.337
Chlorinated water	3.0×10^3	1.0×10^3	1.1×10^3	0.405
Filtered water	7.0×10^4	3.2×10^4	3.1×10^4	0.434

4.4 Solar disinfection

The number of *Salmonella* before exposure to sunlight were 1.6×10^5 , 2.9×10^5 and 3.3×10^5 in set A, B and C respectively. There was complete absence of *Salmonella* in set A and B after five hours of continuous exposure to sunlight. In set C, the number of *Salmonella* varied with time reaching a maximum of (4.1×10^5) after five hours of exposure (Table 4.10).

The result showed a significantly high level ($F = 22.25$, $d f = 2$, $P = 0.001$) of *Salmonella* colony forming units from set C (20.3×10^5 cfu/100 ml) compared to set B (13.7×10^5 cfu/100 ml) and set A (7.00×10^5 cfu/100 ml). Set B and C served as controls in the experiment. Water turbidity was not significantly different in the three sets of treatments

($P > 0.05$). However, mean temperature in set C (22.0 °C) was lower compared to those of set A (43.7 °C) and set B (43.8 °C).

Table 4.10: Mean *Salmonella* (cfu/100 ml) after solar disinfection in Nakuru North Sub-County from May to September 2012

Set A		Set B		Set C	
Mean cfu/100ml x10 ⁵	Time (Hrs)	Mean cfu/100ml x10 ⁵	Time (Hrs)	Mean cfu/100ml x 10 ⁵	Time (Hrs)
1.6	0	2.9	0	3.3	0
2.2	1	4.4	1	3.6	1
1.8	2	3.5	2	3.0	2
0.9	3	1.5	3	2.8	3
0.5	4	1.4	4	3.5	4
0.0	5	0.0	5	4.1	5

Cfu, colony forming unit; Set A, infected water from the water sources; B, water inoculated with commercial *Salmonella* and C, contaminated water from water sources which was kept in the dark.

4.5 Serotyping of *Salmonella*

Four different serotypes of *Salmonella* were isolated using disk diffusion method (Plate 4.1); 48 (45.7 %) *Salmonella enterica* serovar Typhimurium, 24 (22.9 %) *S. enterica* serovar Typhi, 23 (21.9 %) *S. enterica* serovar Enteritidis and 10 (9.5) *S. enterica* serovar Dublin (Table 4.11). The serovars were isolated from raw milk, intestinal wastes, fruits and vegetable salads, waste water, water from community taps and river Kandutura and treated water samples.

4.6 Antimicrobial sensitivity test

The pure *Salmonella* isolates were tested with seven different antibiotics to establish their levels of resistance to antibiotics (Plate 4.2). Those resistant to nalidixic acid were 96.2 %, amoxicillin 11.4 %, cephalixin 7 %, ciprofloxacin 1.9 % and sulfa-trimethoprim

Table 4.11: *Salmonella* serotypes isolated in Nakuru North Sub-County from May to September 2012

Bacterial species	Frequency	Percent
<i>Salmonella enterica</i> serovar Typhimurium	48	45.7
<i>Salmonella enterica</i> serovar Typhi	24	22.9
<i>Salmonella enterica</i> serovar Enteritidis	23	21.9
<i>Salmonella enterica</i> serovar Dublin	10	9.5
Total	105	100

1.9 %. However, 19 % were intermediate for ciprofloxacin, cephalexin (11.4 %) while in chloramphenicol, amoxicillin and sulfa-trimethoprim was 1.9 % each (Table 4.12). The organisms had a sensitivity of 100 % to gentamycin, chloramphenicol (98.1 %), sulfa-trimethoprim (96.2 %), amoxicillin (86.7 %), cephalexin (81.9 %), ciprofloxacin (79.0 %) and nalidixic acid (3.8 %).

Table 4.12: Antimicrobial sensitivity test results for the seven antimicrobials in Nakuru North Sub-County from May to September 2012

Antimicrobials	No. of isolates tested	Resistant		Intermediate		Sensitive	
		F	%	F	%	F	%
CL30	105	7	6.7	12	11.4	86	81.9
NA30	105	101	96.2	0	0.0	4	3.8
C30	105	0	0.0	2	1.9	103	98.1
CIP5	105	2	1.9	20	19.0	83	79.0
CN10	105	0	0.0	0	0.0	105	100.0
AML10	105	12	11.4	2	1.9	91	86.7
SXT25	105	2	1.9	2	1.9	101	96.2

CL, Cephalexin; NA30, Nalidixic Acid; C30, Chloramphenicol; CIP5, Ciprofloxacin, CN10, Gentamicin; AML10, Amoxicillin; SXT25, Sulfa-trimethoprim and F-frequency.

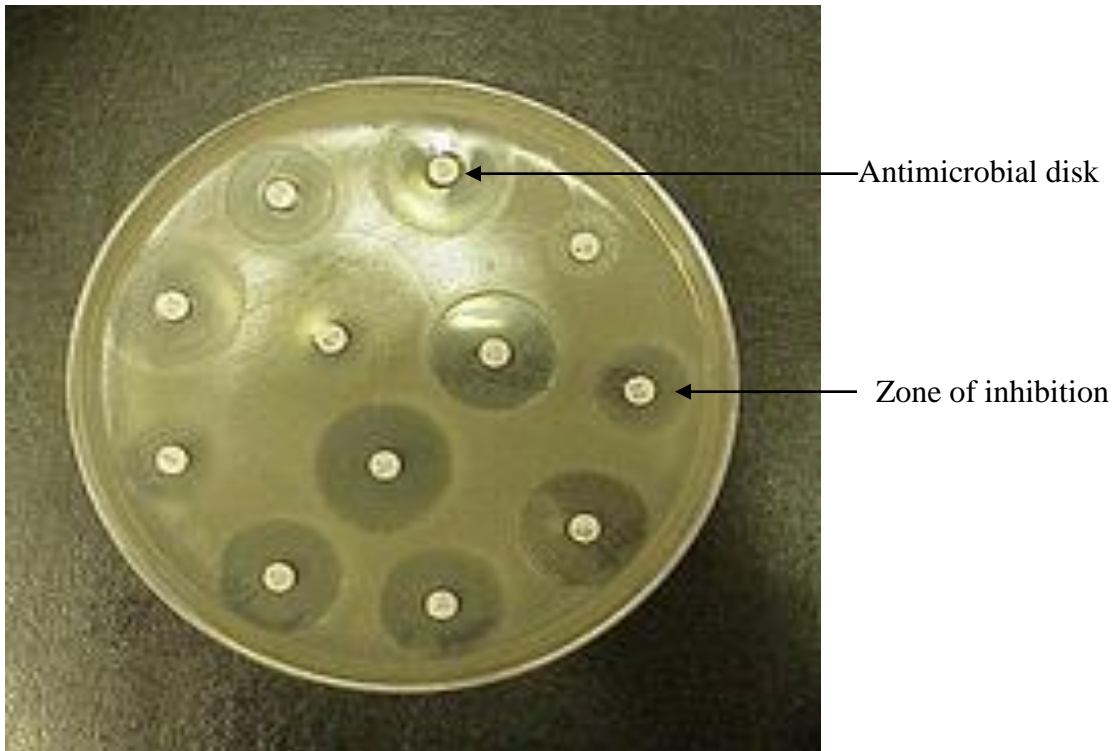


Plate 4.3: Sensitivity test using Karby Bauer disk diffusion test.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Predisposing factors of salmonellosis infections

This study sought to determine whether sex had any effect on transmission of *Salmonella*. Findings from this study indicated that sex was not a risk factor to transmission of *Salmonella*. These findings were contrarily to those obtained in Nigeria where males were more susceptible to salmonellosis infection (Abdullahi, 2010). These results could be associated with the males and females having the same feeding habits. The level of education was found to influence *Salmonella* transmission trend (Table 4.1). These results were consistent with those obtained in Nigeria (Abdullahi, 2010). The infection rate of salmonellosis reduced from people with low education level to those with high levels of education. This was attributed to knowledge obtained through education on personal hygiene which may have reduced the infection rate.

People with full time employment were found to be less prone to salmonellosis compared to other types of occupation with the students having the highest rate of infection (Table 4.1). These results were similar to previously found in Kenya (Jagals and Jagals, 2004) where income had a high influence on infection. This could be attributed to people with a high income being in position to buy improved facilities for waste disposal and health services coupled with having the ability to buy food that has been prepared more safely.

The method of food storage after its preparation was significantly associated with high chances of salmonellosis infection including its covering and cleaning of utensils (Figure 4.2 and table 4.1). These findings were similar to those obtained in Tanzania where 10 % infection occurred in people living in households that didn't cover their food and which also had delayed cleaning of utensils rates (Nusrat *et al.*, 2012). It was stipulated that dust containing these pathogens could settle on uncovered food. In addition, delayed washing of these dishes may have given the bacteria time for multiplication while contaminated water from rivers and community taps could have been used in cleaning the utensils (Lockwood *et al.*, 2006).

On hand washing practices, there was a significant relationship between hand washing and transmission of salmonellosis (Table 4.1). The results of this study were contrary to those obtained in a previous study in Malawi (Fisseha *et al.*, 2006). This could have possibly resulted from a high population pressure in Kenya than in Malawi (Onyeka and Ochieng, 2009).

Human and animal waste disposal posed a risk for transmission of *Salmonella* (Table 4.1). These findings supported those obtained in Tanzania and Addis Ababa where inadequate latrines and improper animal wastes disposal lead to an increase in cases of salmonellosis by 15 % (Curtis *et al.*, 2000; Pruess-Ustun *et al.*, 2004). These may have resulted from defecating in the bushes and use of animal wastes as manures without treatment (Ofor *et al.*, 2009). Moreover, some of these faecal materials might have ended up in rivers leading to further contamination of water sources.

Presence of blocked sewers influenced transmission of *Salmonella* (Table 4.1). These findings were agreed with those obtained elsewhere in Kenya where absence of sewerage system increased (3.2 %) chances of contracting salmonellosis (Clasen *et al.*, 2007). This difference may have resulted from the current study area having adequate sewerage systems. In this study, waste water significantly influenced salmonellosis infection rate (Table 4.1). These results were similar to those obtained in Uganda where waste water was found to increase (5.26 %) cases of salmonellosis (Howard *et al.*, 2004). The explanation for the above results could be that people living in areas with poor waste water disposal get oftenly contaminated by wastes water leading to high rate of infection (Rusin *et al.*, 2000).

People in the study area mainly obtain their water from community piped water taps and river Kandutura. Results from this study indicated that tap and river water used by the residents are at risk of *Salmonella* contamination and subsequent transmission within the community (Table 4.1). These findings were lower compared to those previously obtained in Kisumu, Kenya (Iijima *et al.*, 2001; Lars *et al.*, 2012). This variation in the results may have resulted from differences in the level of sanitation and sewage system management in the two cities (Clasen *et al.*, 2001). However, it was found out that most community water taps had untreated water drawn directly from River Kandutura. This supplied water posed a high risk of infection considering that through its sources and along its path dumping of wastes is a common norm worsened by non existance of a sewage system.

The results found in this study were in contrast with those obtained in South Africa (Iijima *et al.*, 2001; Liang *et al.*, 2008) where methods of water treatment determined transmission of *Salmonella*. However, the presence of *Salmonella* in treated water could have resulted from cross contamination from environmental contaminants after water treatment.

5.1.2 *Salmonella* load of samples

High levels of *Salmonella* were isolated in this study (Table 4.2) compared to those obtained from previous studies in Kenya in bovine milk (9.2×10^2 cfu/100 ml) (Shitandi and Sternesjö, 2004). However, these findings were similar to those obtained in South Africa (Gündoğan *et al.*, 2006; Ateba *et al.*, 2010). Poor hygiene practices especially milking using bare hands and poor farm management practices could have contributed to detected pathogens in milk.

High numbers of *Salmonella* were detected from intestinal wastes of sheep and cattle (Table 4.3 and 4.4). These results were in contrast with those obtained from elsewhere in Kenya (6.3 %), Ghana (33 %) and Nigeria (9.5 %) (Abrahams *et al.*, 2000; Ekperigin and Nagaraja, 2000; Adesiuyn *et al.*, 2001). The different proportions of *Salmonella* loads obtained in these studies may be associated with the differences in time between slaughter and sample collection.

The *Salmonella* counts observed from the fruits and vegetable salads in this study (Table 4.5) were higher than those obtained in other parts of the world (1.0×10^3) (Pradnya and

Patel, 2008; Uzeh *et al.*, 2009; Bukar *et al.*, 2010). The findings suggest that poor storage conditions of the salads together with growing of vegetables with untreated water may have contributed to the high bacterial load. Use of contaminated cutting knives and also infected salad handlers could be a contributing factor to infection.

A recent study on isolation of *Salmonella* from waste water in Dakar, Senegal had lower microbial load (1.0×10^2) compared to those obtained in this study (Ndiaye *et al.*, 2011). The high microbial load obtained in this study (Table 4.6) could be due to storm runoff or waste water from washing of children's clothes which could be potentially contaminated (Amoah *et al.*, 2005).

Previous studies have indicated the presence of *Salmonella* in community taps (2.0×10^5) (Chitrakar and Jackson, 2002; Bhatta *et al.*, 2007). The *Salmonella* isolated from these studies indicated higher values compared to those obtained in this study (Table 4.7). The low isolates obtained in the current study could be due to biofilm formation in the pipes that convey water from the source, the contamination level of the water source or differences in the hygiene level of the study areas. In addition, the sewer lines and waste water drainage pipes were seen lying together with the community portable water pipes which could be another source of contamination.

The microbial contamination obtained from river Kandutura indicated that contamination of water increases progressively from upstream to downstream (Table 4.8). The values obtained in this study were high compared to those obtained from a previous study

carried out in Kenya where $7.3 \times 10^3/100$ ml *Salmonella* were isolated from water in river Njoro (Kiruki *et al.*, 2011). The variation in *Salmonella* isolates from upstream to downstream could have resulted from contamination of the stream where pollution increases from upstream to downstream (Abera *et al.*, 2005). In addition, people could possibly be washing, bathing and drawing water using contaminated cans, including direct drinking water by livestock from same water sources (Birhanu, 2008).

5.1.3 Effectiveness of water treatment methods

The results of *Salmonella* isolation from boiled water (Table 4.9) agree with previous studies that explored the reasons for recontamination of water during collection, boiling, storage, and use at home (Roberts *et al.*, 2001; Wright, 2003) where microbial load reported ranged from $1.0 \times 10^4/100$ ml to $2.0 \times 10^5/100$ ml. The results of the present study could be attributed to inadequate boiling. The results of this study on isolation of *Salmonella* from chlorinated water confirm those obtained by Quik *et al.* (2002). These results could have been caused by people in the study area using sub-optimal amount of chlorine. In using ceramic filters for treatment of water, the method was found to be insufficient (Montgomery and Elimelech, 2007). Poor fabrication of the filtering candles by local manufactures could be a limiting factor.

Solar disinfection was found to be an effective method of treating water (Table 4.10). This is similar to results obtained by Conroy *et al.* (2004) while carrying out a study in Kajiado District in Kenya. However, Oates *et al.* (2003) observed that water has to be left outside for two days for effective disinfection to take place using this method. This

difference could be attributed to the duration of sunny periods within a day and also the season during which the method is used (Ashbolt, 2004).

5.1.4 Serotyping of *Salmonella*

The presence of *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Typhi is of high human health concern because they are responsible for many cases of enteric fever (Fey *et al.*, 2000). The large number of *Salmonella enterica* serovar Typhimurium in this study compared to other serovars agrees with a study carried out elsewhere. The possible reason is the ambiguitors nature of the serovar. The isolation of *Salmonella enterica* serovar Enteritidis confirms studies in different countries in recent years (Bailey *et al.*, 2002). The reason for these results could be that the serovar is responsible for allot of cross contamination in a number of food sources hence its survive rate (Orji *et al.*, 2005). On the other hand, low isolation of *Salmonella enterica* serovar Dublin disagree with a previous study by (Molbak *et al.*, 2002) which could be a consequence of the season in which the two studies were carried out because its prevalence is high during the rainy season.

5.1.5 Antimicrobial resistance patterns of *Salmonella*

The low proportion (6.7 %) of *Salmonella* serotypes that were resistant to cephalexin disagreed with a previous study by Senthilkumar and Prabakaran (2005) where a much higher proportion (34 %) of the isolates were resistant to cephalexin. This could be associated with the reduced usage in the recent past of cephalexin in both animals and human therapy. The *Salmonella* isolates had high (96.2 %) resistance to nalidixic acid

and low (1.9 %) resistance to ciprofloxacin, the only quinolones in this study. This is in agreement with a previous study carried out by Molbak *et al.* (2002). These results may have arisen from the fact that quinolone resistance is chromosomally mediated, thus allowing increased *Salmonella* quinolone resistance in humans and animals (Pezzella *et al.*, 2004). Resistant to chloramphenicol was only observed at the intermediate level at a very low proportion (1.9 %). This was contrary to the findings obtained by Cardoso *et al.* (2006) where no resistance to chloramphenicol was observed.

No resistance was observed to gentamicin which is in agreement with studies carried out in western parts of Kenya (Hart and Kariuki, 2000). The re-emergence of gentamicin and chloramphenicol sensitivity could be attributed to the limited use of the antimicrobials during the last decade (Khan and Shukla, 2004). There was resistance to amoxicillin (11.4 %) which is lower than the 96 % resistance obtained by Lakshmi *et al.* (2006) in India. This could have been caused by lack of laboratories for isolating the bacteria leading to physicians prescribing amoxicillin which is a broad spectrum antibiotic (Okeke *et al.*, 2007). Unlike findings by Tiruneh (2009) in Ethiopia, there was resistance to sulfa-trimethoprim in the present study which can be attributed to the wide usage of the antimicrobial in animal rearing and also in human treatment (Choi *et al.*, 2005).

5.2 Conclusion

This study established that the level of education, occupation, storage of food, cleaning of kitchen utensils, hand washing practices, human waste disposal, animal waste disposal, sewage in the area, waste water disposal, sources of water and water treatment posed a risk to transmission of *Salmonella*. On the other hand, sex and methods of water

treatment did not influence transmission of *Salmonella*. The positive food and water samples collected in Nakuru North Sub-county, from raw milk, intestinal wastes from sheep and cattle, waste water, fruit and vegetable salads, community taps and river water sources are contaminated with *Salmonella*. The samples had *Salmonella* beyond the WHO acceptable levels.

The most effective method of water treatment was chlorination. In addition, solar disinfection is effective upon continuous exposure of water to sunlight for three to five hours. The most prevalent *Salmonella* serotypes in Nakuru North are *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Typhi, *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Dublin. *Salmonella* isolates in Nakuru North Sub-County are sensitive to gentamicin and resistant to cephalexin, nalidixic acid, chloramphenicol, ciprofloxacin, amoxicillin and sulfa-trimethoprim.

5.3 Recommendations

Prevention, treatment and control of salmonellosis infections are necessary before an outbreak occurs. Decision makers should mobilize the community to improve health situations. This can be achieved through, regular inspection of food substances by the authorities concerned to ensure that infected foods are not sold to consumers. Provision of adequate and safe water to the residents of Nakuru North Sub-County is another measure that can go along way in controlling salmonellosis.

Other measures include; health education using local languages related to personal hygiene such as hand washing after visiting the toilet and before handling food, proper food handling and storage, cost effective water treatment methods and proper storage of the treated water. In addition, sensitization of health personnels, veterinary officers and the entire population on prudent use of antimicrobials can help in controlling antimicrobial resistance. Lastly, there's need to conduct a similar study to cover a large population and area.

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APPENDICES**APPENDIX I: CONSENT FORM**

Hello, I am Waithaka Paul Njenga, a masters student of microbiology at Kenyatta University, Nairobi. I am here today to carry out a study on risks factors and control methods of salmonellosis. The information you give is very important and therefore kindly be sincere in your responses. I assure you that the information, will be handled with total confidence and at no time will you be required to identify yourself by name. Kindly answer the questions as completely and as clearly as possible. You are free to choose to participate or not to participate.

Do you agree to participate in the study? No [] Yes []

If Yes,

Signature.....Date.....

APPENDIX II: QUANTITATIVE DATA COLLECTION TOOL (STRUCTURED QUESTIONNAIRE)

(A) Basic information

1) Date of the interview..... 2) Study site.....3) Code of the interview.....

(B) Socio-Demographic information

4) Sex/Gender of the participant 1) Male [] 2) Female []

5) Age in years 1) 18-20 [] 2) 20-29 [] 3) 30-39 [] 4) 40-49 [] 5) 50-59 [] 6) 60+ []

(C) Social-economic information

6) What is/was your main occupation in the last month? 1) Student [] 2) Employed full time [] 3) Employed part time [] 4) Business/self-employed [] 5) Sick leave []

6) Voluntary retirement [] 7) Involuntary retirement [] 8) Unemployed [] 9) others (specify).....

(D) Level of education

7) What's your level of education? 1) None [] 2) Primary education i.e. std 1-8 []

3) Secondary education; form 1-4 [] 4) University/college education [] 5) Adult education []

(E) Storage of food

8) Where do you store your food? 1) In a refrigerator [] 2) At room temperature without covering [] 3) At room temperature with covering []

9) When do you clean your kitchen equipments and utensils be cleaned?

1) After each use [] 2) When you start working on another type of food [] 3) After four hour interval [] 4) Other (specify).....

- 10) Do you disinfect your kitchen equipments and utensils after cleaning them 1) yes []
2) No []

F) Hand washing

- 11) Does washing hands before handling reduce the risk of contamination? Yes [] No []

G) Environmental hygiene

- 12) Where does your family dispose its waste? 1) In the latrine/toilet [] 2) In a nearby bush []

- 13) Do you have animals in your compound Yes [] No [].

If yes, how do you dispose off their waste? 1) In a pit [] 2) Scattered in the compound []

- 3) Used as manure in the farm []

- 14) How often do you have blocked sewers in the neighbourhood?

- 1) None [] 2) Occasionally [] 3) Always []

- 15) How do you dispose your waste water? 1) Pouring outside the house at a central place [] 2) In a pit [] 3) other (specify).....

H) Water hygiene

- 16) What is your water source? 1) A nearby river (stream) [] 2) Well [] 3) Piped water []

- 17) Do you treat your water before using it? Yes [] No []

- 18) If yes, what method(s) of water treatment do you use? 1) Boiling [] 2) Chlorination [] 3) Filtration [] 4) Solar disinfection []