SALMONELLA IN APPARENTLY HEALTHY DOGS: PREVALENCE, SEROTYPES, ANTIMICROBIAL RESISTANCE PROFILE AND PUBLIC PERCEPTION ON PET MANAGEMENT AND ZOONOTIC CANINE DISEASES, IN ADDIS ABABA, ETHIOPIA

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Thesis submitted to the School of Veterinary Medicine, Jimma University, in a partial fulfillment of the requirements for the Masters of Veterinary Public Health (MVPH)

By

Bitsu Kiflu

January, 2016

Jimma, Ethiopia

DEDICATION

This thesis is dedicated to my parents, my brothers and my sister, for their endless love, support and encouragement.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have duly acknowledged. It has been submitted in partial fulfillment of the requirements for M.Sc degree in Veterinary Public Health to Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine. I truly declare that this thesis is not submitted to any other institution anywhere for the award of any academic certificate. Quotations from this thesis are allowable with accurate acknowledgement of source.

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ABBREVIATIONS

AAI	Animal-assisted interventions
ALIPB	Aklilu lemma institute of pathobiology
AMR	Antimicrobial resistance
BGA	Brilliant green agar
BPW	Buffered peptone water
CDC	Center for disease control
CLSI	Clinical and laboratory standards initiatives
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GALT	Gut Associated Lymphoid Tissue
HEA	Hektone enteric agar
KAP	Knowledge, Attitude and Practice
M.A.S.L	meter above sea level
MA	Mega ampere
MDR	Multiple drug resistant
MKTT	Müller-Kauffmann Tetrathionate-novobiocin broth
µl	Micro liter
NTS	Non-Typhoidal Salmonella
OIE	Office International des Epizooties (World Animal Health Organization)
PCR	Polymerase chain reaction
RVS	Rappaport-Vassiliadis soy broth

SPI	Salmonella pathogenicity island
Spp.	species
T3SS	Type III secretion system
TSA	Tryptone soya agar
TSI	Tryiple sugar iron
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholat agar

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ABSTRACT

Owning dogs have benefits such as companionship, physical activity and protection. However the intimate relationship between dogs and their owners has the potential risk of human exposure to bacterial pathogens, such as *Salmonella*. Dogs are heavily associated with asymptomatic carriage of *Salmonella* and there have been several reports on transmission of *Salmonella* from dogs to humans. This study therefore aimed to determine the prevalence and distribution of *Salmonella* serotypes in healthy dogs, to investigate antimicrobial resistance profile and assess public knowledge, attitude and practice on dog related managements and zoonotic canine diseases. A total of 360 faecal swabs were investigated for *Salmonella* shedding by using culture method, PCR, serotyping and phage typing, and the *Salmonella* carriage rate was 42 (11.67%). Fourteen different serotypes were detected and the most predominant were *S.* Bronx (16.67%) and *S.* Newport (14.28%), followed by *S.* Typhimrium (9.52%), *S.* Indiana (9.52%), *S.* Kentucky (9.52%), *S.* Saintpaul (9.52%) and *S.* Virchow (9.52%). Other serotypes were *S.* Anatum (4.76%), *S.* Haifa (4.76%), *S.* Braenderup (2.38%) and *S.* Muenchen (2.38%). *Salmonella* Bronx, *S.* Chailey, *S.* Indiana, *S.* Minnesota and *S.* Tarshyne were reported for the first time in Ethiopia. Statistically significant association ($p<0.05$) was found between *Salmonella* infection status and symptom of diarrhea during the past 60 days, sleeping place of the dogs, cleaning frequency of dog's feces and drinking contaminated water. Antimicrobial sensitivity test was done for a panel of 16 antimicrobials and the result revealed that 18 (42.85%) of the isolates were resistant to one or more antimicrobials tested. Twenty six percent of the isolates were resistant to ampicillin, 23.80% to amoxicillin+clavulanic acid, doxycycline and oxytetracycline. Most of the isolates were susceptible to nalidixic Acid (97.62%), gentamicin (97.62%), kanamycin (97.62%) and ceftriaxone (95.23%). All (100%) of the isolates were susceptible to ciprofloxacin and amikacin. Multiple resistance (to two or more antimicrobials) were detected in 16 (38.1%) of the isolates. All *S.* Newport isolates were multiple antimicrobial resistant and one *S.* Indiana, Two *S.* Saintpaul and one *S.* Haifa isolates were resistant to more than five antimicrobials. The knowledge, attitude and practices of dog owners towards pet husbandry, pet contact and zoonotic canine disease were found insufficient. In conclusion, the current study revealed that asymptomatic carriage rate of *Salmonella* in apparently healthy dogs in Addis Ababa is high, suggesting the possible high risk of infection of human population in close contact with these dogs. Therefore it is important to create public awareness on pet husbandry, zoonotic canine diseases, prevention measures and good hygiene practices.

Key words: Antimicrobial resistance, Dogs, KAP, *Salmonella*, Serotypes, Zoonosis

1. INTRODUCTION

Dogs are known to have been kept as first pets and first domesticated species since prehistoric times (Smith and Whitfield, 2012). Throughout those years, their role in our lives has changed from one of guardian and hunting companion, to farm hand and most recently, pet and family member (Leonard, 2014). There are many potential benefits to having dogs in our lives. Those benefits include companionship, physical activity, protection, improved mental health and stress relief, animal-assisted therapies, and increased independence for those with disabilities (Hodgson and Darling, 2011; Beetz, *et al*., 2012a; Beetz, *et al*., 2012b). However, Close bond between dogs and humans remain a major threat to public health, with dogs harboring a bewildering number of infective stages of disease causative agents transmissible to man and other domestic animals (Stull, 2012).

Dogs can carry numerous zoonotic pathogens (i.e., infections that can spread from animals to humans), including several species of bacteria, parasites, viruses, and fungi. Some of the zoonotic diseases of concern include salmonellosis, campylobacteriosis, giardiasis, rabies, vector-borne zoonoses, including leishmaniasis, zoonotic helminths, and zoonotic dermatoses such as ringworm (Ballweber, *et al*., 2010; Chomel and Sun, 2011; Deplazes, *et al*., 2011; Weese, 2011; Greene, 2012). The presence of dog faeces in urban settings due to the habit of dog owners of not removing dog faeces from the street may represent a problem for hygiene and public health. Dog faeces may contain several types of microorganisms potentially pathogenic for humans. Pathogenic bacteria that cause diarrhea in human including Campylobacter, Salmonella, Yersinia and E. coli can be found in faeces of dogs (Lefebvre *et al*., 2008; Chaban *et al*., 2010).

Bacterial antimicrobial resistance is an increasingly serious concern in both human and animal, which given the close relationship we share with our pets, is yet another zoonotic infectious disease risk that requires thorough study, understanding, and consideration (Guardabassi, *et al*., 2004; Clarke, 2006; Weese, 2008; Umber and Bender, 2009). Until recently, very few studies have investigated the role of pet dogs as a potential source of antimicrobial resistant bacteria in humans. The majority of studies have focused on food animals and farm environments, since food animals are believed to be the major source of

resistant strains of non-typhoidal *Salmonella* (Freitas *et al*., 2010). However, Antimicrobial resistant *Salmonella* isolates, including several multidrug-resistant (MDR) isolates and isolates resistant to cephalosporins and fluoroquinolones have been reported in dogs and other companion animals (Guardabassi, *et al*., 2004; Lloyd, 2007; Umber and Bender, 2009).

Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*Salmonella enterica*, and *S. bongori*). Although primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, human sewage and in any material subjected to faecal contamination (OIE, 2010). Symptomatic presentation of *Salmonella* infection in dogs is rare, however dogs are regarded as one of the more important asymptomatic carriers of *Salmonella,* as they can harbour high numbers of the organism in the intestines and mesenteric lymph nodes, which can be shed in their faeces without symptomatic presentation (Greene, 2006; Finley *et al*., 2007; Hoelzer *et al*., 2011). Investigations have also shown that naturally occurring salmonellosis in dogs can result in a shedding period of up to 7 weeks (Finley *et al*., 2007). This could be of significant importance to public health as dogs have frequent and close contact with family members in households. The key to balancing the benefits and risks of dog ownership is comprehensive consideration of pet-related management factors, such as diet, environmental exposures, proper veterinary care, and owner-related factors, such as immune competency, age and health status. In addition, since dogs share the same environments, foods, and many of the possible infectious disease exposures as people, they are a potentially rich source of information for public health surveillance, including surveillance related to enteric bacteria, emerging infectious diseases, antimicrobial resistance and environmental contaminants (Kile, *et al*., 2005; Gubernot, *et al*., 2008; Moore and Lund, 2009; Schmidt, 2009).

The prevalence of *salmonella* in faecal samples from clinically healthy or hospitalized dogs in USA has been reported to range from 1% to 36%, but it has been suggested that the prevalence in developed countries is probably decreasing because more pets are fed commercially processed foods (Greene, 2006). Recent figures show a prevalence of < 1% in dogs within the midland regions of UK (Lowden *et al*., 2015); < 1% in a UK vet-visiting population (Parsons *et al*., 2009) and a prevalence of 2.1% in household pets and 6.3% in stray dogs in Taiwan (Tsai *et al*., 2007). A number of medically important serotypes for

humans have been isolated from domestic dogs and several studies have reported the isolation of multidrug-resistant isolates (Guardabassi *et al*., 2004).

In Ethiopia, information concerning the epidemiology, diagnosis, treatment and management of canine diseases and those of zoonotic concern is largely scarce. This might be due to a combination of various factors. One of these factors may be agriculture is the means of livelihood for about two-thirds of the work force and emphasis is given to farm animals. Currently, however, people living in the urban areas are increasing and customizing western culture is wide spread among the people. Thus this has resulted in changing attitudes towards keeping companion animals and demanding veterinary services (Gebretsadik *et al*., 2014). Very little attention is given for diseases of pets like dogs and cats in Ethiopia and veterinary researches mainly focuses on large animals. Few studies that have been done so far are more concerned on rabies and zoonotic parasites. In this regard, so far there is no available data on the occurrence of *Salmonella* in dogs found in Addis Ababa as well as in the country as a whole. In addition, very few studies have evaluated the general public's knowledge, attitude and practice towards pet husbandry, contact related attitude and pet-associated zoonoses. The present study would be essential for development and application of control measures on zoonotic disease of companion animals. Therefore, this study was conducted with the following objectives:

- To determine the prevalence and distribution of *Salmonella* serotypes in dogs.
- To determine the susceptibility of *Salmonella* isolates to antimicrobial agents used in veterinary and human *medicine* for the treatment of bacterial diseases.
- To assess community knowledge, attitude and practice (KAP) regarding dog husbandry, dog-contact related attitude, zoonotic diseases knowledge and public health implication of canine salmonellosis.

2. LITRATURE REVIEW

2.1. Human-Companion Animal Bond

Human-Animal-Interactions (HAI) is a young discipline which embraces people's association with all kinds of animals including companion pets, wildlife, therapy, agricultural, zoo and laboratory animals (McCune and Serpell, 2012). The term "human-companion animal bond" is used to describe the relationship between people and their pets, and the influence of this relationship on the psychological and physiological states of these groups (Stull, 2012). Nowadays, interaction with companion animals is more intimate as a large proportion of households keep them indoors and share their living area, including bedrooms (McCune and Serpell, 2012). The numbers of pet animals kept within households are increasing and the range of animal species kept for this purpose has extended from traditional household pets such as dogs and cats to encompass rodents, rabbits, ferrets, birds, amphibians, reptiles and ornamental fish (FEDIAF, 2012). The vast majority of pet owners regard their pets as their friends and/or family members. Dogs are the most common pets, followed by cats, horses, and birds (Froma, 2009).

Companionship with animals has shown an encouraging trend in health benefit, including promoting physical and mental well-being, in all ages of the population. Pets provide social support, and this support acts as a buffer against the stresses of everyday life (Kikusui *et al.*, 2006). People whom share their living environment with a companion animal have shown to have healthier physiological responses to stress indicated by a lower level of cortisol in the blood, self-reported anxiety, lower baseline heart rate and blood pressure. In addition, people demonstrate less cardiovascular reactivity to, and a faster recovery, from mild stressors (Allen *et al*., 2002). Numerous studies highlight physiologic benefits of owning pet. Pet interaction, whether active or passive, tends to lower anxiety levels in subjects, and thus decrease the onset, severity, or progression of stress-related conditions (McConnell *et al*., 2011). Furthermore; it is thought that the reduction in blood pressure achieved through dog ownership can be equal to the reduction achieved by changing to a low salt diet or cutting down on alcohol (Allen *et al*., 2002). Many studies have also addressed the contribution of pets to human psychological well-being. Studies have been done on groups facing stressful

life events such as bereavement, illness, and homelessness. Findings from these studies often indicate that pets play a significant supportive role, reducing depression and loneliness and providing companionship and a need for responsibility (Karen, 2007). A number of studies demonstrate the positive impact of pets on coping with chronic conditions and on the course and treatment of illness such as heart disease, dementia, and cancer (Johnson, *et al*, 2005; Friedmann and Tsai, 2006). Pets also have been found to influence the course and optimal functioning with pervasive developmental disabilities and mental health disorders including schizophrenia, depression, anxiety, and Attention Deficit Hyperactivity Disorder (ADHD) (Karen *et al*., 2007; Beck, 2005).

2.2. Pet Zoonosis (Public Health Implications of Zoonotic Pathogens in Dogs)

Even though Dogs provides numerous physical, social and psychological benefits through their relationships with humans, concerns about the transmission of zoonotic infections between dogs and humans have been raised (Leonard, 2014). Pet-associated bacterial zoonoses represent a relatively neglected area compared with food borne zoonoses. However, the close contact between household pets and people offers favourable conditions for transmission by direct contact (e.g. petting, licking or physical injuries) or indirectly through contamination of food and domestic environments. Indeed, frequent sharing of skin microbiota between people and their dogs has been shown, thus emphasizing the role of contact (Song *et al*., 2013). Zoonoses are of special concern for people who are young, old, pregnant or immunocompromised, and therefore particularly susceptible to infections. Furthermore, young children may be more exposed to bacteria originating from household pets due to lower hygiene standards and closer physical contact with these animals and the household environment (e.g. floors and carpets) (Damborg *et al*., 2015).

Domestic dogs have long been recognized as potential sources of several zoonotic enteric pathogens and oftentimes, dogs are subclinical carriers of these pathogens, making the extent of their true risk to human health difficult to measure (Hackett and Lappin, 2003; Weese and Fulford, 2011). Among zoonotic enteric bacteria, *Salmonella*, worldwide distributed bacteria, are responsible for large numbers of infections in both humans and animals (Acha and Szyfres, 2003). Because of the close contact with human beings, the incidence of *Salmonella*

infections in dogs or the intestinal carriage of *Salmonella* by dogs is very important to public health (Hoelzer *et al*., 2011). However, in companion animal practice, veterinarians tend to be concerned with only a handful of zoonotic diseases, most importantly rabies, parasitic worms (roundworm, hookworm, etc.), dermatophytosis (ringworm), and some bacterial pathogens (Leptospira, Brucella, etc.) (Glickman *et al*., 2006; Mani and Maguire, 2009; Weese *et al*., 2011).

2.3. *Salmonella* morphology and taxonomy

The bacteria *Salmonella* was first discovered by Dr. Daniel Salmon, an American veterinary bacteriologist in 1886 (Bell, 2002). *Salmonella* are Gram-negative, facultatively anaerobic, rod shaped bacteria, typically motile by possession of peritrichous flagella. They belong to the *Enterobacteriaceae* family which also includes pathogens such as *Escherichia coli*, *Shigella* and *Klebsiella* (Craig and James, 2006). Members of the genus *Salmonella* are ubiquitous pathogens that infect a wide variety of mammals, birds, reptiles, and even insects (Greene, 2006). They are capable of survival in a diverse range of environments including water, soil, animal hosts and faeces. *Salmonella* spp. has relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. The growth and survival of *Salmonella* spp. is influenced by a number of factors such as temperature, pH, water activity and the presence of preservatives. *Salmonella* prefers to grow at 37°C (98.6°F), but has the ability to grow at a wide range of temperatures, from 6 to 46°C (43 to 115°F). This provides Salmonella with many opportunities to grow (Danielle *et al*., 2006). At lower temperatures *Salmonella* spp. have the ability to survive long term frozen storage. These organisms can grow in a broad pH range of 4.1–9.0, with an optimum pH range for growth being 6.5–7.5 (Danielle *et al*., 2006). Typically they inhabit the harsh nutrient-limiting conditions of the lower intestinal tract of animal hosts. The capacity to utilize scarce nutrients effectively and efficiently relies on the embedded proteins in the outer membranes where they play a role in transportation of such nutrients (Preena, 2013).

The genus *Salmonella* comprises of two species; *S. enterica* and *S. bongori*. *Salmonella enterica* is associated with causing disease in warm blooded animals including human hosts, whilst *S. bongori*, is frequently associated with the intestines of cold-blooded animals

including snakes and lizards and is not commonly associated with warm blooded animals. *S. enterica* can be further sub divided into six subspecies: *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, S. *enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae* and VI, *S. enterica* subsp. *Indica* (Dieckmann *et al*., 2008).

There are currently over 2,600 *Salmonella* serotypes (Guibourdenche *et al*., 2010), which are serologically identified by antigenic variation in the O (Lipopolysaccharide), H (Flagella) and Vi (Capsular) antigens in accordance with the Kauffmann–White scheme (Grimont and Weill, 2007). Of the total serovars, 99% of them are in *S. enterica* and almost 60% belong to S. *enterica* subsp. enterica (Grimont *et al*., 2007; Guibourdenche *et al*., 2010).

Most of the isolates that cause disease in humans and other mammals belong to S. enterica subsp. *enterica*. Some species of *Salmonellae* show a preference for certain animal hosts, and each domesticated farm animal species appears to have an adapted *Salmonella* species: horse—*Salmonella* abortus equi, cow—S. Dublin, sheep—*Salmonella* abortus ovis, pig—*Salmonella* choleraesuis, and fowl—*Salmonella pullorum* and *Salmonella gallinarum* (Greene, 2006). A few *Salmonella* serovars, such as *Salmonella typhi*, S. *paratyphi* A, S. *paratyphi* B and S. *paratyphi* C, are adapted to humans and cause typhoid fever, a serious and potentially fatal systemic infection. They are transmitted mainly from person to person and have no significant animal reservoirs (Ashish *et al*., 2006). The remaining *Salmonella* serovars, often referred to as non-typhoidal *Salmonella*, show little or no specific host adaptation and are equally pathogenic for human and other animals (CFSPH, 2005). Many have been isolated from vertebrates and invertebrates and the environment. These *Salmonella* serotypes or individual isolates of certain serotypes vary widely in their ability to infect and produce disease within a given animal host (Greene, 2006). Table 2 describes the relative distribution of *Salmonella* species, subspecies and serovars as described by Guibourdenche *et al*. (2010)

Table 1: Organization of *Salmonella* species and subspecies

Species	Subspecies	Number of serovars in each subspecies
Salmonella enterica	*Enterica*	1547
	Salamae	513
	Arizonae	100
	Diarizonae	341
	Houtenae	73
	Indica	13
Salmonella bongori		23

Source: Guibourdenche *et al*. (2010)

2.4. Pathogenesis

As one of the most important foodborne pathogens, entry into the host is typically via the oral route and generally an infective dose of 10^3-10^7colony forming unit are required to cause infection. This variability probably reflects the ability of *Salmonella* to resist the low pH of the stomach a powerful component of host defense (Fuaci and Jameson, 2005). As with other infectious diseases the course and outcome of the infection are dependent upon a variety of factors including inoculating dose, immune status of the host and genetic background of both host and infecting organism (Getenet, 2008) After oral ingestion *Salmonella* has to survive passage through the gastric acid, evade killing by digestive enzymes, bile salts, opsonisation by secretory IgA, defensins and other antimicrobial peptides as well as other innate immune defense mechanisms to gain access to the underlying epithelium and deeper tissues. The gastric acid would normally reduce the inoculum size significantly; however, *Salmonella* have an adoptive acid-tolerance response, which may increase their survival through the stomach. The bacteria that are able to overcome this attach to and penetrate the intestinal wall to reach the Gut Associated Lymphoid Tissue (GALT) (Rikke, 2012).

Adhesion to the intestinal epithelium is generally considered to be the first step in pathogenesis preceding invasion. This is accomplished by an interaction between a host cell factor and adhesins present on the surface of the bacterial cell. *Salmonella* as well as many other of the Enterobacteriaceae generates type 1 fimbriae, the most widely used type of fimbrial mechanism. Fimbriae (pili) are believed to mediate this adhesive process (Min *et al*.,

2012). Moreover, type 1 fimbrials modulate bacterial gut tropism as well. Over 80% of *Salmonella* enterica isolates encode and express this type of fimbriae suggesting that type 1 fimbria plays an important role in some stages of *Salmonella* invasion and life cycle (Chuang *et al*., 2008). The adhesion and invasion process is conferred by the type III secretion system (T3SS) 1, a protein complex that is associated with at least 20 structural and regulatory proteins (Foley and Lynne, 2008). T3SS-1 has a needle like structure and injects protein effectors into the epithelial cell. The translocated proteins cause cytoskeletal rearrangements in the host cell which leads to membrane ruffling and bacterial internalization through macropinocytosis. The internalized bacteria cells reside in a membrane bound vacuole (SCV) in which they cross the epithelia layer (Malina *et al*., 2008).

Following adhesion the pathogens invade by micropincytosis and destroy microfolds cells (M cells), located in the Peyer's patches, which triggers an inflammatory response. Peyer's patches are clusters of mammalian lymph follicles and serve as a main port of entry for *Salmonella* serotypes (Tomomi *et al*., 2008). Once inside the Peyer's patches macrophages and polymorphs are recruited to the site of infection and engulf the pathogenic cells producing intracellular superoxide radicals; which usually leads to intracellular killing (Bhan *et al*., 2005). During systemic infections bacteria spread from the GALT via the efferent lymphatic system and the thoracic duct into the vena cava. From the bloodstream *Salmonella* spread throughout the body (Stephan, 2009).

Non-Typhoidal serotypes multiply in the Peyer's patch tissue where they are drained into the mesenteric lymph nodes. It is essential for successful pathogens to overcome host phagocytes, complement, antibodies and other immunological cellular entities. If the host is unable to contain the infection then clinical gastroenteritis is presented; with diarrhoea and vomiting (NHS, 2011). These clinical symptoms are due to enterotoxins produced by the bacilli and their association with proteins and genes including the SopE protein and stn, respectively (Masayuki *et al*., 2012). As well as patients presenting with gastroenteritis, an immunocompromised host can also develop infections such as enteric fever and bacteraemia. This occurs when the blood-barrier is breeched; the pathogen enters the bloodstream via the thoracic duct which could potentially lead to metastatic foci of intravascular lesions, oesteomyelitis and meningitis, all of which are fatal. When this situation arises, the bacilli are

able to migrate into the spleen, liver, gall bladder, bone marrow, lymph nodes and kidneys where they survive and multiply (Bhan *et al*., 2005).

2.5. Virulence factors

Salmonella express a variety of virulence factors which mediate the organism's pathogenicity. Its virulence is associated with a combination of chromosomal and plasmid factors. These factors may include polymorphic surface carbohydrates, multiple fimbrial adhesins, phase-variable flagella and well-structured mechanisms for invasion and survival in host macrophages and other cells (William. 2013). There are approximately 200 genes including those on the five chromosomal pathogenicity islands (SPI-1 to SPI-5) on *Salmonella* chromosomes that are essential for virulence (Antonio and Olivia, 2009).

The majority of virulence genes of *Salmonella* are clustered in regions distributed over the chromosome called *Salmonella* pathogenicity islands. Until recently more than 10 SPIs has been identified on the *Salmonella* chromosome, but SPI -1 and SPI -2 are the central for pathogenesis of *Salmonella* infections (Yakhya *et al*., 2009). A pathogenicity island is a genetic element that occurs as a distinct and separate unit in the bacterial chromosome. SPI-1 encodes the T3SS-1 that enables *Salmonella* to enter many types of host cells. Its major role is the invasion of the intestinal epithelium as strains that harbor mutations within this gene island are reduced in virulence when administered orally but not when given systemically (Fierer and Guiney. 2007). The acquisition of SPI-1 allowed *Salmonella* to cross the intestinal epithelium and to access a new niche. In the following, *Salmonella* adapted to this new environment by acquiring new genes, such as genes of the SPI-2 (Fierer and Guiney, 2007).

SPI-2 encodes T3SS-2 that is required for survival and growth in macrophages as well as in epithelia cells and that is expressed 2-3 hours post infection. It is induced intracellular, after formation of the *Salmonella* containing vacuole (SCV) as a response to various signals, such as low osmolarity, nutrient depletion and acidification (Foley and Lynne, 2008). It functions in translocating different effector proteins into the cytosol that interact with targets in the host cell ((Antonio and Olivia, 2009). The region of the island encoding the T3SS-2 is present in all *Salmonella* except S. bongori (Fierer and Guiney, 2007).

Other important *Salmonella* spp. virulence factors are found on virulence plasmids. All of the virulence plasmids share a highly conserved region designated spv (*Salmonella* plasmid virulence). The spv region promotes rapid growth and survival of *Salmonella* spp. within the host cells and it is important for systemic infection. The spv genes are thought to facilitate intracellular replication in macrophages and systemic spread. In human macrophages, they appear to induce cytotoxicity (Chishih *et al*., 2008).

2.6. Clinical features of Salmonellosis and treatment

Outcomes of exposure to non-typhoidal *Salmonella* spp. can range from having no effect, to colonization of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonization with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhoea, mild fever, vomiting, dehydration, headache and/or prostration. The incubation period is 8–72 hours (usually 24–48 hours) and symptoms last for 2–7 days (Darby and Sheorey, 2008). Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as arthritis, appendicitis, meningitis or pneumonia as a consequence of infection (FDA, 2012).

Salmonella species are shed in large numbers in the faeces of infected individuals at the onset of illness. In the case of non-typhoid disease, bacterial shedding continues for about 4 weeks after illness in adults and 7 weeks in children. It is estimated that 0.5% of individuals with non-typhoid salmonellosis become long-term carriers and continues shedding the bacteria on an ongoing basis (Crum-Cianflone, 2008).

The disease can affect all species of domestic animals; young animals and pregnant animals are the most susceptible (OIE, 2010). Enteric disease, often presenting as bloody or profuse watery diarrhoea with pyrexia, is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis, necrosis of extremities and respiratory disease, may be seen. The signs and lesions are not pathognomonic. Many

animals, especially poultry and pigs, may also be infected but show no clinical illness (Wray and Wray, 2000). Such animals may be important in relation to the spread of infection between flocks and herds and as causes of human food poisoning. In the latter case, this can occur when these animals enter the food chain thus producing contaminated food products. Wild animals such as badgers and some types of birds may carry specific strains of *Salmonella* (Wray and Wray, 2000).

Serotypes *S*. Typhi and *S*. Paratyphi are particularly problematic in humans as they have the ability of causing life threatening systemic infections (Huang and DuPont, 2005). These serotypes are able to overcome the host's immune system and multiply inside the intestine. Successful migration into the blood stream can lead to a systemic, often deep-seated infection called typhoid fever (Chalkias *et al*., 2008). Symptoms usually develop 1–3 weeks after exposure, and may be mild or severe. They include high fever, malaise, headache, constipation or diarrhoea, rose-coloured spots on the chest, and enlarged spleen and liver (WHO, 2O15).

Treatment is limited yet simple, rehydration with clean drinking water is usually sufficient to remove the bacteria from the site of infection. Antibiotics are not typically administered and indeed may prolong the asymptomatic carrier state thus increasing the risk of further contamination and spread (Ebner and Mathew, 2008). However In some instances the symptoms are much more pronounced and graver, which requires the patient to be treated rapidly with antibiotics, commonly: ampicillin, gentamicin, trimethoprim/sulfamethoxazole, ceftriaxone, amoxicillin, ciprofloxacin, chloramphenicol and co-trimoxazole, although there is increasing evidence of resistance to these commonly administered therapies. In particular, *S*. Typhimurium DT104 (A, C, S, Su, T) has become resistant to many of the frequently prescribed antibiotics including ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. Resistant strains delay treatment which could lead to an exacerbated infection, dire consequences and potentially, cause the death of the patient (HPA, 2011).

2.6.1. *Salmonella* in healthy dogs

Dogs generally seem to be resistant to *Salmonella* infection and most cases are latent and non-clinical (Greene, 2006). However, infections in immunocompromised, stressed, puppies and

very old dogs are manifested by clinical signs likely due to their naive or weakened immune systems, respectively (Morley *et al*., 2006). The majority of infections are asymptomatic. However, gastrointestinal disease manifested as enterocolitis and endotoxemia can occur and is often associated with diarrhea, fever, vomiting, anorexia, dehydration and depression (Greene, 2006). Meningoencephalitis, respiratory distress and conjunctivitis have also been described (Carter and Quinn, 2000). Infected bitches can suffer from further complications including miscarriage, stillbirth, utero infections and weak offspring, but these occurrences are rare (Carter and Quinn, 2000; Greene, 2006). Furthermore, gastroenteritis can cause acute enteritis in the intestinal tract evident from blood-stained faeces. Histological reports have demonstrated that the pathogenesis of *Salmonella* in dogs can cause mucosal erosion and infiltration of neutrophiles and macrophages into the lamina propria surrounding the Peyer's patches, as in human salmonellosis. Infection is dependent upon many factors, specifically, infectious dose, serotype or strain, the virulence of the strain and bacterial competition, or lack of it, within the gut flora (Carter and Quinn, 2000). Though Symptomatic presentations are rare, dogs regarded as asymptomatic carriers of *Salmonella* has shown long periods of intermittent shedding through faeces for up to 6 weeks and this is clearly of public health concern (Finley *et al*., 2007).

Appropriate therapy for canine salmonellosis varies according to the type and severity of clinical illness. Treatment of acute infections tends to be by replenishment of water and electrolytes losses from vomitus and diarrhea. Fluids can be administered orally when vomiting is not a problem (Greene, 2006). Antimicrobial intervention is deemed undesirable to avoid exacerbating the issue of antibiotic resistance of bacteria. However, systemic infections, due to their severity, require antimicrobial treatment, typically: trimethoprim-sulfonamide combinations, ampicillin, flouroquinolones, Amoxicilin and third generation cephalosporins. Aminoglycosides such as gentamicin and amikacin may be considered when bacterial resistance is anticipated, but the risk of renal toxicity precludes their routine use (Greene, 2006). The selection of antibiotics is based upon antimicrobial sensitivity patterns and their availability. Conversely, in some cases it has been observed that clinical signs shown by dogs are treated swiftly and blindly with a broad spectrum of antibiotics as diagnosis is sometimes considered too time consuming. This questionable prescribing practice

contributes to the growing number of *Salmonella* isolates being resistant to available antibiotics (Preena, 2013).

2.7. Source of infection and transmission of *Salmonella* in dogs

Salmonella is found in the environment and the gastrointestinal tract of wild and farmed animals. Animals may become infected with *Salmonella* through environmental contamination, other animals or contaminated feed. Both animals and humans can function as *Salmonella* reservoirs. In addition to sheep, goats, cattle, chickens and pigs, other animals which can become infected with *Salmonella* include geese and other birds, lizards and other reptiles, shellfish, and amphibians such as turtles. Indeed, most *Salmonella* contamination is of animal origin (Carlos *et al*., 2012). Among livestock production systems, *Salmonella* is more frequently isolated from poultry (chicken, turkey, duck, and pheasants) than from other animals (Freitas *et al*., 2010). *Salmonella* has also been isolated from insects, raw meats, factory surfaces and domestic kitchen utensils (Braoudaki and Hilton, 2004). The vast diversity of environments that could be potential sources of *Salmonella* gives rise to comprehensive routes of transmission which contributes to the success of the pathogen. It is generally regarded that the presence of *Salmonella* within other environments can be sourced back to some prior faecal contamination (Preena, 2013).

One of the major routes of *Salmonella* infection is via contaminated human food, often meat and dairy products from farm animals (Jayarao *et al*., 2006). The pathogenic cells may harbour in undercooked food or food that is not washed thoroughly before consumption. In addition, it is not solely direct farm animal contact that is the source of infection but also the food, water and environment they inhabit. The transmission route of *Salmonella* infection is complex and difficult to control as there are many areas of exposure, including interaction with pets and animals (Preena, 2013).

Salmonella-infected animals shed the microorganism in the feces from where it can spread into soil, water, crops and/or other animals. All *Salmonella* serotypes can be harbored in the gastrointestinal tract of livestock. The most common chain of events leading to this foodborne illness involves healthy carrier animals which subsequently transfer the pathogen to humans

during production, handling and/or consumption. *Salmonella* transmission to food processing plants and food production equipment is a serious public health issue (Pawin and John, 2006).

Dogs are potentially at high risk of contracting *Salmonella* as they employ indiscriminate eating habits largely associated with scavenging and hunting for small animals including rodents, wild birds, insects and reptiles. This tendency, coupled with the ubiquitous nature of *Salmonella*, increases the exposure of the microorganism to the dogs. Major sources of *Salmonella* contamination tend to be linked to contaminated water and food, associated heavily with meat products (mainly chicken), offal and meat bone meal as the frequency of contamination within these food sources is relatively high (Carter and Quinn, 2000; Morley *et al*., 2006; Finley *et al*., 2008). However, transmission in dogs has been reported to be largely associated with the faecal-oral route via carrier animals and their faeces; this route of transmission is exacerbated by the copraphagic tendencies of many dogs (Carter and Quinn 2000; Finley *et al*., 2006). Livestock exposure (e.g., cattle, pigs, horses, and poultry) or contact with their feces or contaminated environments may lead to increased *Salmonella* exposure in pet dogs, as these animals have been shown to commonly carry *Salmonella* (LeJeune and Hancock, 2001).

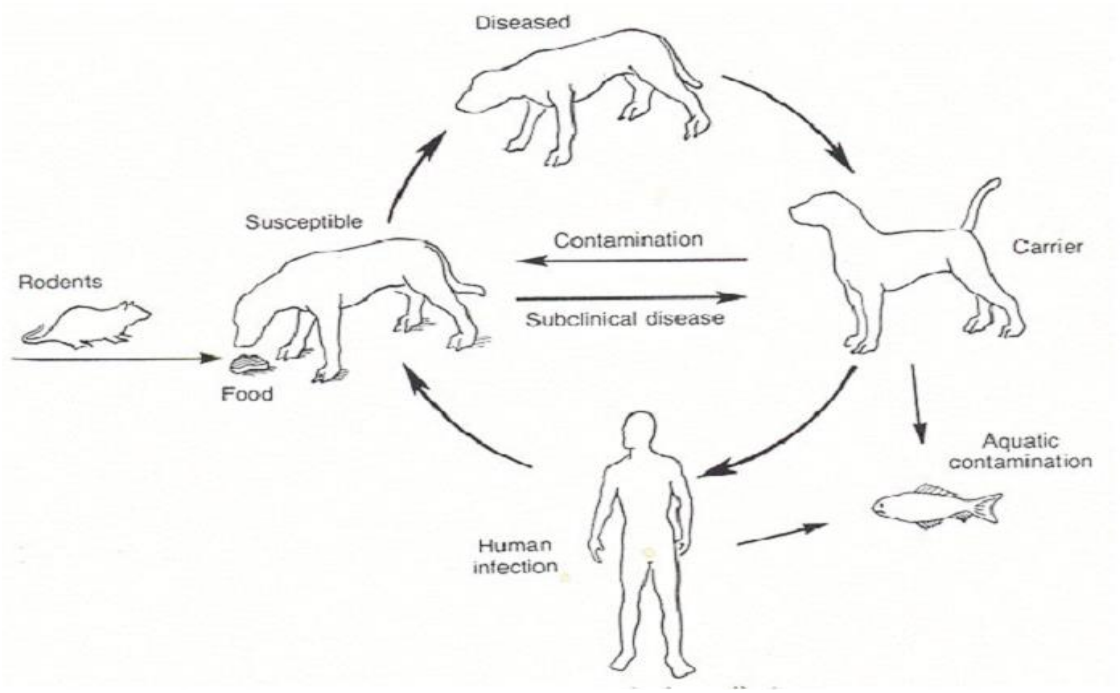

Figure 1: Pathways of *Salmonella* spp. transmission (Greene, 2006)

2.8. Isolation and identification of *Salmonella*

Salmonella can be isolated either from tissues collected aseptically at necropsy or from feces, rectal swabs, environmental samples, food products and feedstuffs. Individual samples for bacteriological tests should be collected as aseptically as possible by following the respective standards. Moreover, precaution should be taken to avoid cross contamination of samples during transport and at the laboratory. Packages should also be kept cool and accompanied by adequate information (OIE, 2010).

More rapid immunological and molecular screening methods have been devised to detect *salmonella*. However, Culture based identification methods are the most widely used detection techniques and remain the gold standard for the detection of *Salmonella* due to their selectivity and sensitivity. Depending on the approach, standard culture methods typically require 5–7 days to obtain a result as they rely on the ability of *Salmonella* to multiply to visible colonies, which can then be characterized by performing additional biochemical and or serological tests (Joseph and Carlos, 2012).

The international standard method for detection of *Salmonella*, (ISO 6579:2002), consists of non-selective pre-enrichment in Buffered Peptone Water (BPW), selective enrichment in Rappaport-Vassiliadis with soy broth (RVS) and Müller-Kauffmann tetrathionate-novobiocin broth (MKTTn), plating on the selective solid medium Xylose Lysine Desoxycholat agar (XLD) and an additional plate medium of choice such as Brilliant Green agar (BGA) and a final biochemical and serological confirmation (ISO-6579:2002; OIE, 2010).

2.8.1. Pre-enrichment

The viability state of *Salmonella* is dependent on the specimen presented for analysis, which are typically from hostile environments (Preena, 2013). *Salmonella* may be present in small numbers in environmental samples, faces, animal feed and food and are often accompanied by considerably larger numbers of other *Enterobacteriace* or other families. Cultural methods typically involve the enrichment of a portion of the sample to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock (Smith *et al*., 2013). Therefor it is necessary to use pre-enrichment media to assist the isolation. Furthermore, pre-enrichment is necessary to

permit the detection of low numbers of *Salmonella* or injured *Salmonella* (ISO-6579:2002). Buffered peptone water is the pre enrichment broth of choice which is recommended by ISO-6579. Buffered peptone water is inoculated at ambient temperature with the test portion, and then incubated at 37 °C ± 1 °C for 18 h - 24 h. (ISO-6579, 2002).

2.8.2. Selective enrichment

Success in isolating *Salmonella* is usually enhanced by the inoculation of incubated pre-enrichment broth into selective enrichment media (Joseph and Carlos, 2012). Enrichment media are liquid or semi-solid agar media that contain additives that selectively permit *salmonellae* to grow while inhibiting the growth of other bacteria. Examples of selective enrichment media are sodium tetrathionate, as in Müller–Kaufman broth, selenite F, selenite cysteine, brilliant green broth and Rappaport–Vassiliadis broths, or semi-solid Rappaport Vassiliadis medium. To diminish the risk of obtaining false negative results it is advantageous to use more than one selective enrichment (ISO-6579: 2002; OIE, 210).

Rappaport-Vassiliadis is currently recommended for *Salmonella* recovery from low and highly contaminated foods or faecal samples (Bakr *et al*., 2008). Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth) are inoculated with the culture obtained in pre-enrichment. The RVS broth is incubated at 41.5 °C ± 1 °C for 24 h ± 3 h, and the MKTTn broth at 37 °C ± 1 °C for 24 h ± 3 h (ISO-6579, 2002).

2.8.3. Plating out on Selective Agar

Plating media employ a selective role for the target bacteria and an inhibition of competitor bacteria. Selective media are designed to differentiate *Salmonella* from other species according to their appearances on the agar. They inhibit growth of bacteria other than *Salmonella* and give information on some of the principal differential biochemical characteristics usually non lactose fermentation and hydrogen sulphide (H_2S) production. The results are read after 24 and 48 hours of culture at 37°C (OIE, 2010). Selective plating media is a comprehensive area and choice of selection is typically dependent on cost and specimen

sample. Each agar is designed with a specific mode of action and modifications of established agars are constantly being made for better and reliable confirmation (Preena, 2013).

The most commonly used media selective for *Salmonella* are xylose-lysine-desoxycholate (XLD) agar, *Salmonella* Shigella agar, Bismuth sulfite agar, Hektoen enteric (HE) medium and brilliant green agar. All these media contain both selective and differential ingredients and they are commercially available. Several agar media containing chromogenic substrates for *Salmonella*-specific enzymes have recently been developed and become commercially available. The chromogenic substrates specifically enable *Salmonella* spp. to hydrolyze these substrates and producing well colored Salmonella colonies, clearly differentiating from non-Salmonella colonies (Vera *et al*., 2005). Rambach agar (RAM) was designed to exploit *Salmonella* ability to produce acid from propylene glycol and the selective nature is derived from the presence of bile salts (Preena, 2013).

2.8.4. Confirmation

Following selective agar plating, confirmation will be done by using different biochemical tests whether the colonies resembling *Salmonella* on selective Medias are really *Salmonella*. For confirmation, it is recommended that at least five colonies be identified on selective plates. If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies. Streak the selected colonies onto the surface of non-selective agar plates, such as nutrient agar in a manner which will allow well-isolated colonies to develop. Incubate the inoculated plates at 37 °C ± 1 °C for 24 h ± 3 h and pure cultures is used for biochemical confirmation (ISO-6579,2002; Hendriksen, 2003; WHO, 2010).

The ISO-6579, 2002 standard recommends using the following biochemical tests; TSI agar, Urea agar (Christensen), L-lysine decarboxylase, β-galactosidase (ONPG), Voges Proskauer and Indole tests in this order. From a pure culture on nutrient agar plates, inoculate the media on TSI, Urea agar, L-lysine decarboxylation medium and the LDC control medium, ONPG medium, VP medium, Tryptone medium for indole and Incubate all biochemical tests at 37^{o}C for 18 to24 hours (overnight). Except for VP, which need 48h of incubation (Hendriksen, 2003; WHO, 2010).

Serotyping- In 1926, Bruce White developed the analysis of somatic and flagella antigens, which in 1961 was expanded by Fritz Kauffman to distinguish more than 2000 serovars. In 1980, the nomenclature of today (The Kauffman-White Scheme) was proposed, and is currently maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (Grimont and Weill, 2007).

Salmonella serotyping is based on the immunoreactivity of the two surface antigen groups, the O antigens (somatic antigens) and the H antigens (flagellar antigens) and to a lesser extent the Vi antigens (capsular antigens) which are present in very few serotypes (S. *Typhi,* S. *Paratyphi* C and *S.* Dublin) (Corcoran, 2013). The O antigens are the carbohydrate (O subunits or polysaccharides) component attached to the core oligosaccharide of the lipopolysaccharides molecule. The O antigens can be divided into two groups the O-group antigens (core antigens) and the ancillary antigens (additional polysaccharide subunits that are added to the core antigen structure). Each O antigen has been designated a number for identification. Strains that do not express O antigens are referred to as rough in the antigenic structure details (CDC, 2011). Historically some were assigned alphabetically and the terms are still in use (CDC, 2011). All O antigens detected on the surface are listed sequentially following the White-Kauffman-Le Minor scheme (Grimont and Weill, 2007).

The H antigen is the filamentous portion of the flagellar component of the bacterium. The antigenic differentiation is related to diversity in the middle portion of the flagellin protein. Most *Salmonella* cells can express two different H antigens (diphasic). The phase 1 antigen is encoded by the fliC gene and the phase 2 antigen is encoded by the fliB gene (CDC, 2011). Most cells only express 1 antigen at a single time. Cells that can only express 1 antigen are referred to as monophasic, which can occur naturally in some serovars or through loss of either the fliC gene or fliB genes in serovars that are usually diphasic such as *S.* Typhimurium (Corcoran, 2013).

Antigen-antibody complexes are formed (agglutination) when a bacterial culture is mixed with a specific antiserum directed against bacterial surface components. The complexes are usually visible to the naked eye which allows for easy determination of O and H antigens by

slide agglutination (SSI, 2013). The O antigen is detected in a bacterial suspension taken from an agar plate. Detection of H antigens is performed on a bacterial suspension from broth (to ensure motility of the bacterium). The presence of antigens is detected by agglutination tests on glass slides with the corresponding commercial anti-sera (Corcoran, 2013).

Phage typing- Bacteriophages (phages) are viruses that can only grow or replicate within a bacterial cell (Corcoran, 2013). Phage typing is based on the specificity of a given phage for its host bacterium, and this relationship allows one to use known phages to identify their specific hosts. It can differentiate between strains of the same serotype based on the principle that certain phages will only lyse particular strains of a specific serotype. The lysis pattern can be compared to a standard scheme for each serovar to determine the phage type of the strain (Lappe *et al*., 2009). Therefore, phage typing of *Salmonella* isolates is based on the sensitivity of a particular isolates to a series of bacteriophages at appropriate dilutions. This can be useful to determine whether isolates, which come for different places at different times, are similar or different in their reactions with specific sets of phages used for typing (Rabsch, 2007).

2.9. Antimicrobial Resistance

Antimicrobial resistance is a very complex problem involving various bacterial species, resistance mechanisms, transfer mechanisms and reservoirs. Resistance to antimicrobials and particularly multidrug resistance is an emerging problem in *Enterobacteriaceae* for developing and developed countries (Schwarz and White, 2005). Several studies have shown that inappropriate antibiotic use in food animals is significant threat to human health, as pathogenic-resistant organisms propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products (Garofalo *et al*.,2007; Ramchandani *et al*., 2005). Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans (Mena *et al*., 2008; Diarrassouba *et al*., 2007). In United States it has been reported that most of the antibiotics produced are fed to farm animals as growth promoters and to obtain a better meat to feed ratio (Goldman, 2004). In the pork and poultry industry low levels of bacitracin, chlortetracycline, erythromycin, lincomycin, neomycin, oxytetracycline, penicillin, streptomycin, tylosin or virginiamycin are

administrated in each ton of feed. Over the time these low doses of antimicrobials confer the ability of microorganisms to evolve mechanisms of defense, therefore making them less susceptible to the effect of the drug and contributing to treatment failure (Timothy *et al*., 2102).

The use of antimicrobials is important for the control and treatment of *Salmonella*. However, since the early 1990s, antimicrobial and multidrug resistant *Salmonella* strains have merged, leading to treatment failure (Gong *et al*., 2013). Multidrug-resistant bacteria pose a severe threat to public health, particularly those that are resistant to β-lactams and fluoroquinolones (Lai *et al*., 2014). The increasing number of multidrug-resistant *Salmonella* strains is a global concern, with some countries and international organizations creating surveillance systems which include collaboration between human health, veterinary, and food-related sectors to monitor the spread of these and other foodborne bacteria. Examples include the Danish Integrated Antimicrobial Resistance Monitoring and Research Program, the European Food Safety Authority, the National Antimicrobial Resistance Monitoring System in the USA, and the Global Foodborne Infections Network run by the World Health Organization. These surveillance systems are also employed to monitor antimicrobial resistance, antimicrobial consumption in livestock, and serotype distribution, and data describing the current trend of increasing resistance to multiple drugs has been made available (ECDC, 2013). In contrast, surveillance reports are unavailable in most developing countries. Travel, migration, and the distribution of food between countries can also contribute to the spread of foodborne diseases and multidrug-resistant bacteria. Therefore, monitoring the distribution of *Salmonella* serotypes and levels of antibiotic resistance in animals and animal-food products is also important for maintaining safe travel and the commercial trade in food animals (Lai *et al*., 2014; Russell *et al*., 2014).

2.10. Public health implications of antimicrobial resistant bacteria in companion animals

Antimicrobial resistance bacteria in animals are a health concern globally, and companion animals are a potential reservoir for resistant bacteria (Guardabassi, *et al*., 2004; Boerlin and Reid-Smith, 2008; Acar and Moulin, 2012). Due to a lack of information about antimicrobial resistant bacteria in pets, such as cats and dogs, the true role of companion animals in the

spread of resistant bacteria to humans is poorly understood (Wright, *et al*., 2005; Lloyd, 2007; Weese, 2008). Antimicrobial resistant bacteria in companion animals is difficult to study, due to a lack of surveillance and routine testing (Lloyd, 2007; Murphy, *et al*., 2009).

It is estimated that 70-90% of antimicrobials sold in many developed countries are used in animals, with the majority of this amount being used in food animal production, but the proportion used in companion animals is generally unknown (FDA, 2011; FDA, 2012b). Not only are antimicrobials commonly used in human medicine also used in companion animals, but antimicrobials used in animals which might not be used in human medicine may belong to the same classes of antimicrobials used to treat human infection. This relationship between antimicrobials used in animals and humans is a concern due to the correlation of resistance that can be seen to different drugs within the same class (Guardabassi, *et al*., 2004; Clarke, 2006; Johnson, *et al*., 2006; Skurnik, *et al*., 2006). Recent studies showed that close contact between humans and their pet can lead to the exchange of pathogenic bacteria, including those carrying antibiotic resistant genes (Johnson *et al*., 2006). Various longitudinal retrospective studies have reported an increase in the prevalence of antimicrobial resistance in different zoonotic bacterial species isolated from pet animals (Guardabassi, *et al*., 2004)

2.11. Control and Prevention

Food is clearly a major *Salmonella* infection vehicle. This vital role in salmonellosis outbreaks calls for strict measures to minimize transmission, such as appropriate animal husbandry and agriculture practices, protection of feeds and water from contamination, adequate waste disposal methods and an overall effort to maintain a clean environment around food from farm to fork. Additionally, much of the risk posed by *Salmonella* can be mitigated through proper handling and correct food safety practices, including thorough washing and disinfection. Because foods of animal origin may be contaminated with *Salmonella*, people should not eat raw or undercooked eggs, poultry, or meat. Persons also should not consume raw or unpasteurized milk or other dairy products. Produce should be thoroughly washed. Hands, cutting boards, counters, knives, and other utensils should be washed thoroughly after touching uncooked foods. Hand should be washed before handling food, and between handling different food items (CDC, 2015b).

Prevention of salmonellosis in dogs and cats can be frustrating because of the tendency of some animals to develop a chronic subclinical carrier state or latent infection. Non-typhoid *salmonella* that infect pets are also harbored by many other animals and persist in the environment, making eradication difficult (Greene, 2006). However, thorough cooking of the animals' food, particularly meat and poultry products, good hygiene and the use of heat-processed commercial food products would eliminate major sources of *Salmonella* for dogs and cats. Uneaten, moist food should not be allowed to remain in food bowls at ambient temperatures for long periods, as *Salmonella* may be able to replicate in the food (Carter and Quinn, 2000).

Dogs should be excluded from premises where food is prepared, stored or served. Animals should not be allowed to eat from the same food dishes as humans. Individuals handling dogs and cats must be made aware of the need to thoroughly wash their hands after contact with animals, especially before serving or consuming food (Carter and Quinn, 2000). Continuous monitoring and generation of data on *Salmonella* and salmonellosis outbreaks, and improved surveillance measures are also vital to controlling this public health hazard and to evaluate the magnitude of the problem in each country, locate the origins of outbreaks, and adopt methods designed to reduce risks (Carlos *et al*., 2012).

3. MATERIALS AND METHODES

3.1. Description of the study area and study period

The study was conducted for 10 months from January to October, 2015 in Addis Ababa, which is the capital city and administration center for the Federal Democratic Republic of Ethiopia. Addis Ababa lies 9º 1′48´´North and 38º 44′ 24´´ East (AACG, 2012), it lies in the central highlands of Ethiopia at an altitude of 2500 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26ºC and 11ºC, respectively; with an overall average of 18.7ºC. Highest temperatures are reached in May. The main rainy season extends from June to mid-September. Addis Ababa has a relative humidity varying 70% to 80% during the rainy season and 40% to 50% during the dry season (NMSA, 2012). The city is divided in to 10 sub-cities (Kifle Ketemas) delineated on the basis of geographical set up, population density, asset and service providers' distribution and convenience for administration. The sub-cities are also divided in to woredas, which are the smallest administrative unit in the city. There are 116 woredas in the city administration (AACG,2012).

Figure 2: Map of Addis Ababa Sub-cities (kifle ketemas). Source: (AACG, 2004).

3.2. Study Animals

The study was conducted on apparently healthy dogs which includes all age groups and both sexes. Sick dogs and/or dogs treated with antimicrobials were not included in the study. Faecal samples were obtained from dogs brought to veterinary clinics for vaccination against rabies and from vaccination campaigns at the selected Sub-cities (kifle ketemas) and door to door faecal samples were also collected from volunteer households. Dogs included in this study belongs to four kifle ketemas; Gulele, Arada, Kirkos and Yeka.

3.3. Study Design

A cross-sectional study including microbiological analysis, antimicrobial susceptibility test and questionnaire interview was conducted to determine the prevalence, distribution and antimicrobial resistance profile of *Salmonella* species from dogs and to assess the household knowledge, attitude and practice of dog owners towards their management, pet contact and associated zoonoses in Addis Ababa.

3.4. Sample of size determination

Sample size was determined using the formula described by Thrusfield (2007) with assumption of 50% prevalence in 95% confidence interval.

$$N=1.96^2\, p_{exp}(1-p_{exp})/d^2$$

Where N= required sample size, p=expected prevalence d= desired absolute precision

Therefore, the calculated sample size was 384. However due to unwillingness of some owners and exclusion criteria (being diseased and treated with antimicrobial drugs), only 360 faecal samples were collected and processed. Of the total 360 dogs included in the study, 78 were from Arada, 137 from Gulele, 62 from Yeka and 83 from Kirkos kifle ketemas.

3.5. Sampling procedure and sample collection

Four vetrinary clinics (Gulele, Arada, Yeka and Kirkos) were selected from the 10 governmental veterinary clinics which are found in each kifle ketemas based on the intensity

of dogs number which came for vaccination. Samples were also taken during vaccination campaigns in the selected kifletemas. Door to door samples were also taken from different households by collaboration with health extension officers and private mobile veterinary clinics. Systematic random sampling method was employed both at clinics and vaccination campaigns proportionally to each kifle ketemas.

Rectal swab samples were collected with sterile cotton swab by rotating the swab inside the rectum of the dog and the swab was placed into test tubes containing 10 ml sterilized buffered peptone water (BPW) (Becton Dickinson, France), breaking off the left over wooden shaft pressing against the inside of the test tubes and disposed leaving the cotton swab in the test tubes (Figure 3).

Then the test tubes were sealed, properly labeled and transported to Microbiology Laboratory of Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University in ice box immediately.

Figure 3: Sample collection

3.6. Questionnaire

Faecal samples from dogs were accompanied by a questionnaire, except for samples taken from antirabies campaigns because we were unable to administer questionnaire at the campaign. Owners of dogs were interviewed using a questionnaire that focused on assessing

the possible risk factors of *Salmonella* infection, the owner's knowledge, attitude and practices towards dog management, dog contact-related attitude, sanitation practice and their knowledge on canine zoonotic disease. An example of the dog owner questionnaire is presented in Annex 3.

3.7. Laboratory analysis

3.7.1. Bacteriological Investigations

The technique recommended by the International Organization for Standardization ISO-6579 (2002) was employed in order to isolate and identify *Salmonella* organisms (Annex 1).

Upon arrival of laboratory, the sample were manually homogenized by using vortex mixer (Vortex genie, USA) for approximately 30 seconds and the test tubes were incubated at 37ºC for 24 h.

3.7.1.1. Selective Enrichment

Following incubation, enrichment broths, Rappaport-Vassiliadis soya peptone (RVS) (Oxoid, England) and Müller Kauffman Tetrathionate broth (MKTT) (Oxoid, England) were prepared by following the instructions of the manufacturers (Annex 4). Following this, 1 ml and 0.1 ml aliquot of the sample in pre-enrichment broths were transferred aseptically into 10 ml of Tetrathionate and 10 ml of Rappaport-Vassilliadis broths respectively, mixed with vortex mixer and then incubated for overnight (18 to 24 hours) at 37ºC and 42ºC, respectively.

3.7.1.2. Isolation and Identification

Three selective agars, Xylose Lysine Desoxycholate (XLD) (Oxoid, England), Hektoen Enteric (HE) (Oxoid, England) and Brilliant Green Agar (BGA) (Difco Becton Dickinson, USA) plates were prepared aseptically according to the manufacturer's recommendation (Annex 4).

Following incubation, a loopful of RVS and MKTT suspension was inoculated onto the surface of: XLD, HE and BGA and incubated at 37ºC for 24 hours. Then the plates were analyzed for colony morphology typical of *Salmonella*.

Typical colony of *Salmonella* grown on XLD plates has a slightly transparent zone of reddish color and a black center (H_2S); a pink-red zone may be seen in the media surrounding the colonies (ISO-6579, 2002). Typical *Salmonella* colonies on a BGA agar plate cause the color of the medium to be red/pink (phenol red is the indicator). The colonies are grey-reddish/pink and slightly convex. *Salmonella* typically produce clear colonies with distinct black centers (H_2S) on HEA. If growth was slight or if typical colonies of *Salmonella* were not present, the plates were re-incubated for a further 18 to 24 hours and re-examined for the presence of typical *Salmonella* colonies. Typical *Salmonella* growth on this medias was marked with a + in the record keeping sheets (Annex 2) (Hendriksen, 2003; ISO-6579, 2002).

Two or more *Salmonella* suspect colonies were picked from the selective media and plated onto non-selective media plates (Nutrient agar or Tryptone soya agar) for biochemical confirmation of *Salmonella* and serotyping.

3.7.2. Biochemical identification

All suspected *Salmonella* colonies were picked from the selective media and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, lysine iron agar (LIA), Simmon's citrate agar, urea agar, and incubated for 24 hours at 37ºC. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide production, positive for lysine (purple color), negative for urea hydrolysis (yellow Color or no change) and positive for citrate utilization were considered to be *Salmonella*-positive (ISO-6579, 2002; Mikoleit, 2014).

3.7.3. Polymerase Chain Reaction (PCR)

Each isolates were cultured on tryptone soya agar plate and incubated for 24 h at 37^{o}C. Then pure colony was picked and suspended in 100 µl nuclease-free water (UltraPure DNase/RNase-Free Distilled Water, Thermo Fisher, USA) in PCR tubes. Extraction of DNA was performed by boiling the suspension for 5 min at 95ºC in thermo cycler, centrifuged at 6000 rpm for 5min. and an aliquot (1 µl) of the supernatant was used as template for PCR amplification.

Salmonella genus specific oligonucleotide primers of 25 bp were used to amplify a 496 bp region of histidine transport operon gene of *Salmonella* (Cohen *et al*., 1993). The sequences for the forward and reverse primers from 5' to 3' end were ACTGGCGTTATCCCTTTCTCTGGTG; and ATGTTGTCCTGCCCCTGGTAAGAGA respectively.

PCR Reaction mix (20 µl) consisted 18 µl nuclease-free water, 1 µl of the template DNA, 0.5 µl of each of primers (reverse and forward) was prepared and added into lyophilized PCR premix (AccuPower Taq PCR preMix Bioneer, Korea) tubes which contains 1U Taq DNA polymerase, 250 µM dNTPs (dATP, dCTP, dGTP, dTTP), 1x reaction buffer with 1.5 mM $MgCl_2$, trace amount of tracking dye and patented stabilizer which were already aliquoted in PCR tubes (Annex 4) and Dissolved and spun down. The mixture was then amplified using thermal cycler (VWR, USA) with an initial denaturation (4 min at 95ºC) followed by 30 cycles of denaturation (30 sec at 95ºC), annealing (30 sec at 60ºC) and elongation (45 sec at 72ºC) and final extension for 5 min at 72 ºC.

Detection of PCR Products: For gel electrophoresis, Agarose gel was prepared using 2% agarose made in 1x TAE buffer containing red gel (2.5 µl). After loading the Amplicons in each wells, an electric current of 150 MA and 100 V was applied for about 40 min. Positive results were indicated by the presence of a 496-bp band seen on the gel with an ultraviolet illuminator and taking the gel image using Benchtop 2UV transilluminator. Negative control and known positive control were also placed along with the samples.

3.7.4. *Salmonella* Serotyping and phage typing

Those isolates confirmed as *Salmonella* were sub-cultured on tryptone soya agar slant and shipped to WHO *Salmonella* reference laboratory, Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada, for serotyping and phage typing. The somatic (O) antigens were determined by slide agglutination tests (Ewing, 1986) and flagellar (H) antigens were determined using a microplate agglutination technique (Shipp and Rowe, 1980). The antigenic formulae of Grimont and Weill (2007) were used to identify and assign the serotypes of the isolates. Phage typing of *S*. Typhimurium isolates was performed by the methods developed by Callow (1959) and extended by Anderson *et al*.

(1977) with reference phages obtained from the Public Health England, Gastrointestinal Bacteria Reference Unit, Colindale, England and the Public Health Agency of Canada, National Laboratory for Enteric Pathogens, Winnipeg, Canada. *Salmonella* isolates that reacted with the phages but did not conform to any recognized phage type were designated atypical (AT).

3.7.5. Antimicrobial Susceptibility testing

The antimicrobial susceptibility testing of the isolates were performed for panel of 16 antimicrobials using Kirby-bauer disk diffusion test according to the Clinical and Laboratory Standard Institute guideline (CLSI, 2012). From each isolate, four to five well-isolated colonies grown on Tryptone soya agar were aseptically transferred into test tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37ºC for 4 h or until it achieved the 0.5 McFarland turbidity standards. Then the turbidity of the broth suspension was adjusted by comparing it with 0.5 McFarland turbidity standards **(**Remel, USA) by placing the tubes in front of a McFarland Equivalence Turbidity Standard visual comparison card (Remel, USA). After adjusting the turbidity, sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxoid, England). The plates were held at room temperature for 15 min to allow drying. Then Antibiotic discs with known concentration of antimicrobials were placed on the Muller Hinton agar plate with flamed forceps, inverted and incubated with agar side up for 16-18 h at 37ºC. Following incubation, the diameter of zone of inhibition was measured with a ruler to the nearest millimeter by holding the plate a few inches above a black non-reflecting surface. Interpretation of the results (i.e. categorization of isolates into susceptible, intermediate or resistant) is done according to CLSI guidelines (CLSI, 2012). Reference strain of Escherichia coli ATCC 25922 was used as a quality control (Hendriksen, 2003).

The *Salmonella* isolates were tested for their antimicrobial susceptibility against the following antimicorbials (Sensi-Discs, Becton Dickinson and Company, USA): Amikacin (AN), Amoxicilin/clavulanic acid (AMC), Ampicillin (AM), Cefoxitin (FOX), Ceftriaxone (CRO), Cephalothin (CF), Chloramphenicol (C), Ciprofloxacin (CIP), Doxycycline (DO),

Gentamicin (GM), Kanamycin (K), Nalidixic acid (NA), Neomycin (N), Streptomycin (S), Sulfamethoxazole-Trimethoprim (SXT) and Oxytetracycline (T). The list of antimicrobials used, their symbols and zone of inhibition interpretation to categorize an isolate as susceptible, intermediate or resistant are shown in Table 3

Table 2: List of antimicrobial drugs used for antimicrobial susceptibility test and zone of inhibition interpretation standards

NAME OF DRUG	CODE	Disc Potency	Zone of Inhibition Interpretation Standard (mm)		
			Resistant	Intermediate	Susceptible
Amikacin	AN	30 µg	≤ 14	15-16	≥ 17
Amoxicilin/clavulanic acid	AMC	20/10 µg	≤ 13	14-17	≥ 18
Ampicillin	AM	10 µg	≤ 13	14-16	≥ 17
Cefoxitin	FOX	30 µg	≤ 14	15-17	≥ 18
Ceftriaxone	CRO	30 µg	≤ 19	20-22	≥ 23
Cephalothin	CF	30 µg	≤ 14	15-17	≥ 18
Chloramphenicol	C	30 µg	≤ 12	13-17	≥ 18
Ciprofloxacin	CIP	5 µg	≤ 15	16-20	≥ 21
Doxycycline	DO	30 µg	≤ 10	11-13	≥ 14
Gentamicin	GM	10 µg	≤ 12	13-14	≥ 15
Kanamycin	K	30 µg	≤ 13	14-17	≥ 18
Nalidixic acid	NA	30 µg	≤ 13	14-18	≥ 19
Neomycin	N	30 µg	≤ 13	13-16	≥ 17
Streptomycin	S	10	≤ 11	12-14	≥ 15
Sulfamethoxazole-Trimethoprim	SXT	23.75/1.25 µg	≤ 10	11-15	≥ 16
Oxytetracycline	T	30 µg	≤ 11	12-14	≥ 15

BBL™ Sensi-Disc™ Susceptibility Test Discs, Becton Dickinson and Company, USA

3.8. Data Management and Analysis

The collected data were computed by using statistical package for social science (SPSS version 20.0). Descriptive statistics, graphs and charts were computed for all variables. The associations between *Salmonella* occurrence and categorical factors were compared using person's χ^2 test. This test was followed by binary logistic regression, to account for confounding variables and interactions. The point prevalence was calculated as the number of infected individuals divided by the number of individual's sampled × 100. A P value < 0.05 was reported as statistically significant.

4. RESULTS

4.1. Prevalence of *Salmonella*

Of the total 360 faecal samples examined, 47 were presumed to be *Salmonella* positive by biochemical confirmation, and then subjected to genus specific PCR for confirmation. Forty-two (11.67%) of them were PCR positive for *Salmonella* while the rest five were non-*Salmonella* isolates (Figure 4).

Table 3: Demographics of the sampled dogs included in the study

		Numbers examined (percent)	**No of positive (percent)**	**X^2(P-value)**
kifle ketema's	Gulele	137 (38.05)	16 (11.67)	2.073 (0.56)
	Arada	78 (21.67)	7 (8.97)	
	Kirkos	83 (23.05)	13 (15.66)	
	Yeka	62 (17.22)	6 (9.67)	
Sex	Male	291(80.8)	35 (12.02)	0.192 (0.66)
	Female	69 (19.2)	7 (10.14)	
Age	Puppy (< 6 month)	73 (20.27)	11 (15.01)	4.63 (0.33)
	> 6 to 24 months	84 (23.33)	9 (10.71)	
	> 24 to 72 months	137 (40.83)	16 (11.67)	
	> 72 to 120 months	46 (12.77)	2 (4.34)	
	Above 120 months	20 (2.77)	4 (20)	
Breed	Local	124 (34.44)	12 (9.67)	2.49 (0.29)
	Exotic	174 (48.33)	25 (14.37)	
	Cross	62 (17.22)	5 (8.06)	
Purpose of dog ownership	Guard	130 (36.11)	19 (14.61)	2.93 (0.23)
	Hobby	86 (23.88)	6 (6.97)	
	Guard and hobby	144 (40)	17 (11.80)	
Overall		360 (100)	42 (11.67)	

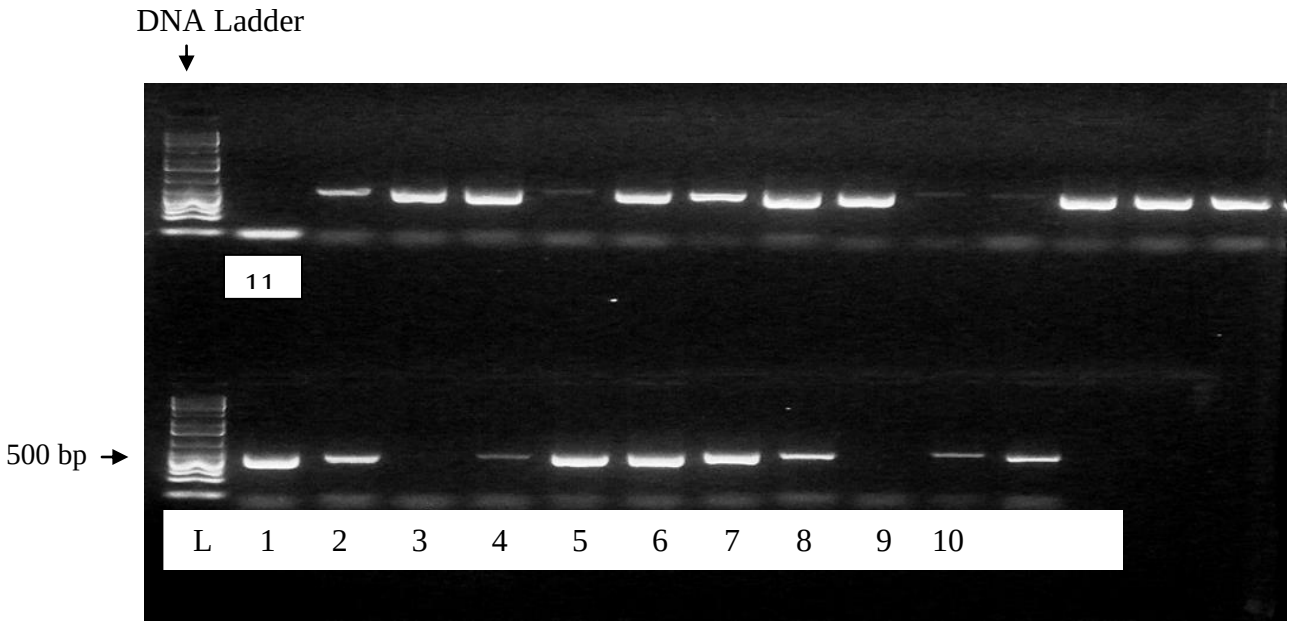

Figure 4: Representative gel image showing results of electrophoresis of products of the PCR reaction. A 496-bp band is seen in each lane with the product of the PCR for *Salmonella* species; lane 1=Positive control; Lanes 2 4, 5, 6, 7, 8 and 10 are positive for *Salmonella*, while Lanes 3 and 9 are negative. Lane L=DNA ladder and lane 11=negative control

4.2. Assessment of the effects of different risk factors considered for the presence of *Salmonella* in dogs.

In total, 18 variables relating to the dogs' health, diet and common exposures were examined in univariable models and 5 were found to be significant at the 5% level (Table 5). An association of *Salmonella* carriage with the risk factors was assessed using logistic regression analysis as well as Chi square test. Simple bivariate and multivariate analysis was used to determine which risk factors were associated with the risk for presence of *salmonella* in dogs and statistically significant associations was seen between *Salmonella* presence and the presence of diarrhea (loose stools) in the last 60 days, sleeping place of the dogs, cleaning frequency of dog's feces and drinking rain water and water from toilet bowls (which is used either to flush the toilet or to wash hands).

Infection with *Salmonella* was observed to be more common (OR = 3.783, 95% CI=1.760-8.132) among dogs which have diarrhea within the past two months than those with no sign of diarrhea.

The result of simple bivariate analysis showed a high risk of *Salmonella* infection in dogs which drinks rain water and water from toilet bowls than those who drink only clean

municipal water. Those who drink rain waters are five times (OR= 5.111, 95% CI =2.344-11.145) more likely to be carrier of *Salmonella* than those who drink only clean water. *Salmonella* is four times more likely to occur in dogs who drink water from toilet bowls (OR=4.086, 95% CI =1.764-9.463) than those who drink clean water only.

Strong association is demonstrated between *Salmonella* infection rate and the place where the dog sleep. Dogs which sleep outside the compound are 11.43 times (95%CI= 2.477-52.720) more likely to be a carrier of *Salmonella* than those who sleep inside their house and inside living room. Dogs which sleep outside their house are 10.182 times (95% CI=2.55-40.68) more likely to be infected with *Salmonella* than those who sleeps inside their house or inside living room.

Table 4: Result of logistic regression and chi square analysis for factors potentially associated with asymptomatic carriage of *Salmonella* among dog owners in Addis Ababa

Variables or Risk factors	Categorical parameters	Total sampled N (%)	Prevalence N (%)	OR(95%CI)	Pearson X^2 (p-value)
Diarrhea in the last 60 days	Yes	62 (24.6%)	16 (25.8%)	3.783 (1.76-8.13)	12.745 (0.000)
	No	190 (75.4%)	16 (8.4%)		
Where the dog does sleep	Living room	68 (27%)	4 (5.9%)	-	23.812 (0.000)
	Dogs house	135 (53.6%)	14 (10.4%)	1.85(0.58-5.86)	
	Living room+dogs house	19 (7.5%)	2 (10.5%)	1.88(0.32-11.16)	
	Outside the house	18 (7.1%)	7 (38.9%)	10.18 (2.55-40.67)	
	Outside the compound	12 (4.8%)	5 (41.7%)	11.43 (2.47-52.72)	
Rain water	Yes	52 (20.6%)	16 (30.8%)	5.11(2.34-11.14)	19.3 (0.000)
	No	200 (79.4%)	16 (8%)		
Water from toilet bowls	Yes	36 (14.3%)	11 (30.6)	4.09(1.76-9.46)	12.081 (0.001)
	No	216 (85.7%)	21 (9.7%)		
Cleaning frequency of the dogs faeces	Daily	154 (61.1%)	14 (9.1%)	-	15.658 (0.008)
	3-5 times weekly	29 (11.5%)	2 (6.9%)	0.74 (0.16-3.34)	
	Weekly	27 (10.7)	4 (14.8%)	1.74 (0.53-5.75)	
	Monthly	17 (6.7%)	3 (17.6%)	2.14 (0.55-8.37)	
	Every three month	10 (3.97%)	4 (40%)	6.67 (1.68-26.48)	
	Never	15 (5.95%)	5 (33.3)	5 (1.50-16.70)	

Many other potential risk factors (Age, Sex, Breed, the place where the dog spent the majority of its time, foods the dogs eat, origin of the meat, weather the dog see a vet regularly or not and sanitation of the dog) were assessed using chi square and logistic regression for their association with the occurrence of *Salmonella*, but no statistically significant association could be demonstrated between them ($P > 0.05$).

4.3. Serotyping and phage typing

From the total of 42 *Salmonella* isolates, 14 different serotypes were recovered. The most prevalent *Salmonella* serotype isolated were *Salmonella* Bronx (16.67%), followed by *S.* Newport (14.28%), *S.* Typhimurium (9.52%), *S.* Indiana (9.52%), *S.* Kentucky (9.52%), *S.* Saintpaul (9.52%) and *S.* Virchow (9.52%). Other serotypes such as *S.* Anatum, *S.* Braenderup, *S.* Chailey, *S.* Haifa, *S.* Minnesota, *S.* Muenchen and *S.* Tarshyne were also identified (Table 6). To our knowledge, *S.* Bronx, *S.* Chailey, *S.* Indiana, *S.* Minnesota and *S.* Tarshyne are reported for the first time in Ethiopia.

Table 5 : *Salmonella* serotypes isolated from healthy dogs in Addis Ababa

Serotypes	Antigens	Phage type	Numbers Isolated (%)
S. Bronx	6,8:c:1,6		7 (16.67)
S. Newport	6,8:e,h:1,2		6 (14.28)
S. Indiana	4:z:1,7		4 (9.52)
S. Kentucky	8,20:i:z6		4 (9.52)
S. Saintpaul	4:e,h:1,2		4 (9.52)
S. Virchow	6,7:r:1,2		4 (9.52)
S. Anatum	10:e,h:1,6		2 (4.76)
S. Haifa	4:z10:1,2		2 (4.76)
S. Typhimrium	4,5:i:1,2	Atypical	2 (4.76)
S. Typhimrium	4,5:i:1,2	74	2 (4.76)
S. Braenderup	6,7:e,h:e,n,z15		1 (2.38)
S. Chailey	6,8:z4,z23:[e,n,z15]		1 (2.38)
S. Minnesota	21:b:e,n,x		1 (2.38)
S. Muenchen	6,8:d:1,2		1 (2.38)
S. Tarshyne	9,12:d:1,6		1 (2.38)
Total			42 (100)

4.4. Antimicrobial Resistance profile

Of the 42 *Salmonella* isolates subjected to antimicrobial susceptibility test using a panel of 16 different antimicrobials (Table 7), 18 isolates (42.86%) which belongs to *S.* Typhimurium, *S.* Tarshyne, *S.* Haifa, *S.* Chailey, *S.* Anatum, *S.* Newport, *S.* Bronx, *S.* Kentucky, *S.* Haifa, *S.* Saintpaul and *S.* Indiana were resistant to one or more of the antimicrobials tested. Among the antimicrobials tested, the highest level of resistance was observed against ampicillin (26.20%), amoxicillin+clavulanic acid (23.80%), doxycycline (23.80%) and oxytetracycline (23.80%). A high percent of susceptibility was exhibited in the isolates towards nalidixic Acid (97.62%), gentamicin (97.62%), kanamycin (97.62%), ceftriaxone (95.23%) and chloramphenicol (92.86%). All isolates were susceptible to ciprofloxacin and amikacin. Fifty percent, 30.95% and 23.81% of the isolates showed intermediate resistant (susceptibility) to neomycin, oxytetracycline and Streptomycin respectively. Multiple antimicrobial resistances (to 2 or more antimicrobials) were recorded in 38.1% of the isolates and resistance to 3 or more antimicrobials was detected in 33.3% of the total isolates, while resistance to 4 or more antimicrobials was detected in 23.8% of the isolates (Table 9). All *S.* Newport isolates were multiple resistant to three or more antimicrobials and one serotype of *S.* Indiana was resistant to seven antimicrobials. Likewise, two *S.* Saintpaul and one *S.* Haifa isolates were resistant to more than five antimicrobials tested. All *S.* Bronx (except one) serotypes were pansuceptible to all antimicrobials tested (Table 8).

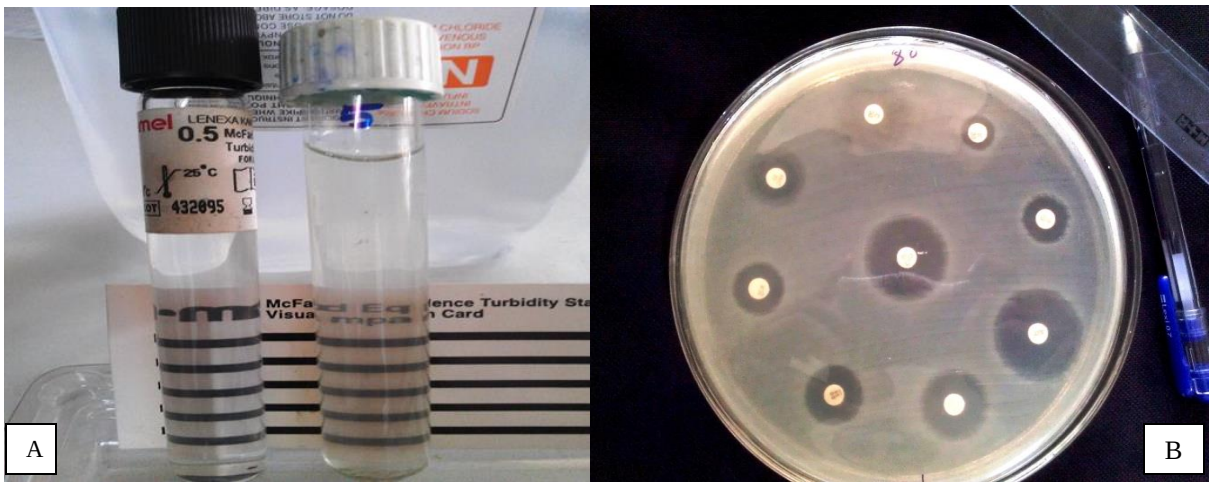

Figure 5: Antimicrobial sensitivity test results (A=comparing the suspension with 0.5 McFarland standards; B= antimicrobial zone of inhibition)

Table 6: Number of susceptible and resistant isolates by antimicrobials

Type of Antimicrobial Agent	Number of isolates		
	Resistant (%)	**Intermediate (%)**	**Susceptible (%)**
Amikacin (AN)	-	-	42 (100)
Amoxicillin-clavulanic acid (AMC)	10 (23.80)	3 (7.14)	29 (69.04)
Ampicillin (AM)	11 (26.20)	-	31 (73.80)
Cefoxitin (FOX)	4 (9.52)	-	38 (90.47)
Ceftriaxone (CRO)	1 (2.38)	1 (2.38)	40 (95.23)
Cephalothin (CF)	7 (16.67)	5 (11.90)	30 (71.42)
Chloramphenicol (C)	3 (7.14)	-	39 (92.86)
Ciprofloxacilin (CIP)	-	-	42 (100)
Doxycycline (DO)	10 (23.80)	5 (11.90)	27 (64.28)
Gentamicin (GM)	1 (2.38)	-	41 (97.62)
Kanamycin (K)	-	1 (2.38)	41 (97.62)
Nalidixic acid (NA)	-	1 (2.38)	41 (97.62)
Neomycin (N)	-	21 (50)	21 (50)
Streptomycin (S)	6 (14.29)	10 (23.81)	26 (61.90)
Sulfamethoxazole-Trimethoprim (SXT)	4 (9.52)	-	38 (90.48)
Oxytetracycline (T)	10 (23.80)	13 (30.95)	19 (45.24)

Table 7: Antimicrobial resistance profile of *Salmonella* serotypes isolated from healthy dogs in Addis Ababa

Serotype	No.	CF	FOX	AM	GM	NA	AMC	T	CIP	K	S	SXT	C	AN	N	DO	CRO
S. Anatum	2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0
S. Braenderup	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. Bronx	7	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0
S. Chailey	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
S. Haifa	2	1	1	0	0	0	1	1	0	0	1	1	0	0	0	1	0
S. Indiana	4	1	1	1	0	0	1	0	0	0	1	1	1	0	0	0	0
S. Kentucky	4	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
S. Minnesota	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. Muenchen	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. Newport	6	2	0	4	0	0	4	5	0	0	3	0	0	0	0	4	0
S. Saintpaul	4	1	0	3	0	0	3	3	0	0	0	1	1	0	0	3	0
S. Tarshyne	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
S. Typhimurium	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S. Virchow	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	42	7	4	11	1	0	10	10	0	0	6	4	3	0	0	10	1
(%)	100	16.7	9.52	26.2	2.38	0	23.8	23.8	0	0	14.3	9.52	7.14	0	0	23.8	2.38

AN=Amikacin, AMC=Amoxicillin-clavulanic acid, AM= Ampicillin, FOX=Cefoxitin, CRO=Ceftriaxone, CF=Cephalothin, C=Chloramphenicol, CIP=Ciprofloxacilin, DO=Doxycycline, GM=Gentamicin, K=Kanamycin, NA=Nalidixic acid, N=Neomycin, S=Streptomycin, SXT=Sulfamethoxazole-Trimethoprim, T=Oxytetracycline

Note: Intermediate antimicrobial resistant profile is not included in this table

Table 8: Multiple antimicrobial resistances profile of *Salmonella* serotypes

Number of antimicrobials to which isolates were Resistance	Antimicrobial resistance pattern (No.)	*Salmonella* Serotypes (No.)	Total No. of isolates (%)
Zero	Pansusceptible	*S.* Bronx (6)	24 (57.14%)
		S. Virchow (4)	
		S. Indiana (3)	
		S. Typhimurium (3)	
		S. Kentucky (3)	
		S. Anatum	
		S. Muenchen	
		S. Braenderup	
		S. Saintpaul	
		S. Minnesota	
One	DO (1)	*S.* Typhimurium	2 (4.76%)
	C (1)	*S.* Tarshyne	
Two	T, DO (1)	*S.* Haifa	2 (4.76%)
	SXT, CRO (1)	*S.* Chailey	
Three	T, S, DO (3)	*S.* Newport (2)	4 (9.52%)
		S. Anatum	
	CF, AM, AMC, (1)	*S.* Newport	
Four	AMC, T, S, DO (1)	*S.* Newport	6 (14.28%)
	CF, AM, AMC, T (1)	*S.* Newport	
	AM, AMC, T, DO (2)	*S.* Newport	
		S. Saintpaul	
	CF, FOX, AM, GM (1)	*S.* Bronx	
	CF, FOX, AM, AMC, (1)	*S.* Kentucky	
Five	CF, FOX, AMC, S, SXT (1)	*S.* Haifa	2 (4.76%)
	CF, AM, AMC, T, DO (1)	*S.* Saintpaul	
Six	AM, AMC, T, SXT, C, DO (1)	*S.* Saintpaul	1 (2.38%)
Seven	CF, FOX, AM, AMC, S, SXT, C (1)	*S.* Indiana	1 (2.38%)

4.5. Questionnaire Result (KAP)

4.5.1. Dog husbandry

The study showed that majority of the owners (70.2%) doesn't take their dog to veterinary service on regular basis (at least once a year) and 85.7% of them don't give their dogs any medicine for a disease (Table 10).

Information was also collected regarding dietary habits of participating dogs. Owners were asked questions regarding their feeding and watering habits of their dogs. Most (92.1%) of the owners feed their dogs a meat, of which 59.05% of them feed a raw meat, 34.48% of dog owners feed their dog cooked meat, while 6.46% of them feed both raw and cooked meat. 91.3 of the dogs eat table scraps/human foods and only 3.6% of the dogs eat commercial pet foods.

79.3% (184) of the owners obtained the meat from local unlicensed markets and only 19.8% (46) of the owners obtains the meat from licensed butchers which sell meats for human consumption. A very small number of owners 2 (0.86%) obtained the meat from supermarkets. All the respondents reported that they give a clean municipal drinking water for their dogs. Besides the clean water they provided, 20.6% and 14.3% of the dogs drink rain water and water from toilet bowls respectively (Table 10).

Twenty seven percent of the respondents reported that they allow their dogs to sleep in their living room and about half (48%) of dog owners, allowed their dog to have contact, sit and/or sleep on their furniture. More than ninety percent of the respondent's did not see their dogs eating birds, lizards/frogs and rats.

Table 9: Pet husbandry

Variables		Yes, N (%)	No, N (%)
Sit and/or sleep on furniture		121 (48)	131(52)
See a Veterinarian on a regular basis (at least once a year)?		75 (29.8)	177 (70.2)
Did you give the dog any medicine for diseases?		36 (14.3)	216 (85.7)
Diarrhea in the last 60 days		62 (24.6)	190 (75.4)
Dog spend the majority of its time	All the time in the house	154 (61.1)	
	Mostly in the house	68 (27)	
	Half inside	17 (6.7)	
	Mostly outside	13 (5.2)	
Dog sleeps	living room	68 (27)	
	Dogs house	135 (53.6)	
	living room and dogs house	19 (7.5)	
	Outside the house	18 (7.1)	
	Outside the compound	12 (4.8)	
What do you feed your dog?	Meat	232 (92.1)	20 (7.9)
	Table scrap/human food	230 (91.3)	22 (8.7)
	Commercial food	9 (3.6)	243 (96.4)
What type of meat did you feed?	Raw	137 (59.05)	
	cooked	80 (34.48)	
	Raw and cooked	15 (6.46)	
Where did you obtain the meat?	Local market	184 (79.31)	
	Licensed butcher	46 (19.8)	
	Super market	2 (0.86)	
Did your dog eat?	Birds	13 (5.2)	239 (94.8)
	Lizard/Frogs	16 (6.3)	236 (93.7)
	Rat	18 (7.1)	234 (92.9)
Did your dog drink?	Rain water	52 (20.6)	200 (79.4)
	Water from the toilet bowl	36 (14.3)	216 (85.7)

4.5.2. Knowledge, attitudes and practices related to pet contact and sanitation of the respondent

Most (84.1%) of the respondents have experience of touching their dogs on regular basis, with 51.6% of them had mouth contact with dogs either for themselves or other member of their family; 38.1% of them reported that at least one member of their family had a contact with feces of the dogs.

Close contact between dogs and children was often reported, as 27% of respondents stated the dog slept in a living room including child's bed room. Owners were asked if they (any member of their family) wash their hands after having contact with the dogs and 78.77% of them wash their hands after having contact with their dogs. Of those respondents who wash their hands, 34.13%, 35.32% and 30.53% of them wash always, usually and sometimes respectively. Twenty one percent of the respondents never wash their hands after having contact with their dog (Table 11).

Table 10: Sanitation and Pet contact-related attitude of respondents

Variables		Yes, N (%)	No, N (%)
Touching the dog		212 (84.1)	40 (15.9)
Having mouth contact with any part of the dog		130 (51.6)	122 (48.4)
Touching or having any contact with the dogs faces		96 (38.1)	156 (61.9)
Do you (any member of family) wash their hands after touching the dog(s)?		167 (78.77)	45 (21.23)
How often?	Always	57 (34.13)	
	Usually	59 (35.32)	
	Sometimes	51 (30.53)	
Do any of the children (member of your family) play in the same area as where the dog(s) go to the bathroom?		115 (45.6)	137 (54.4)

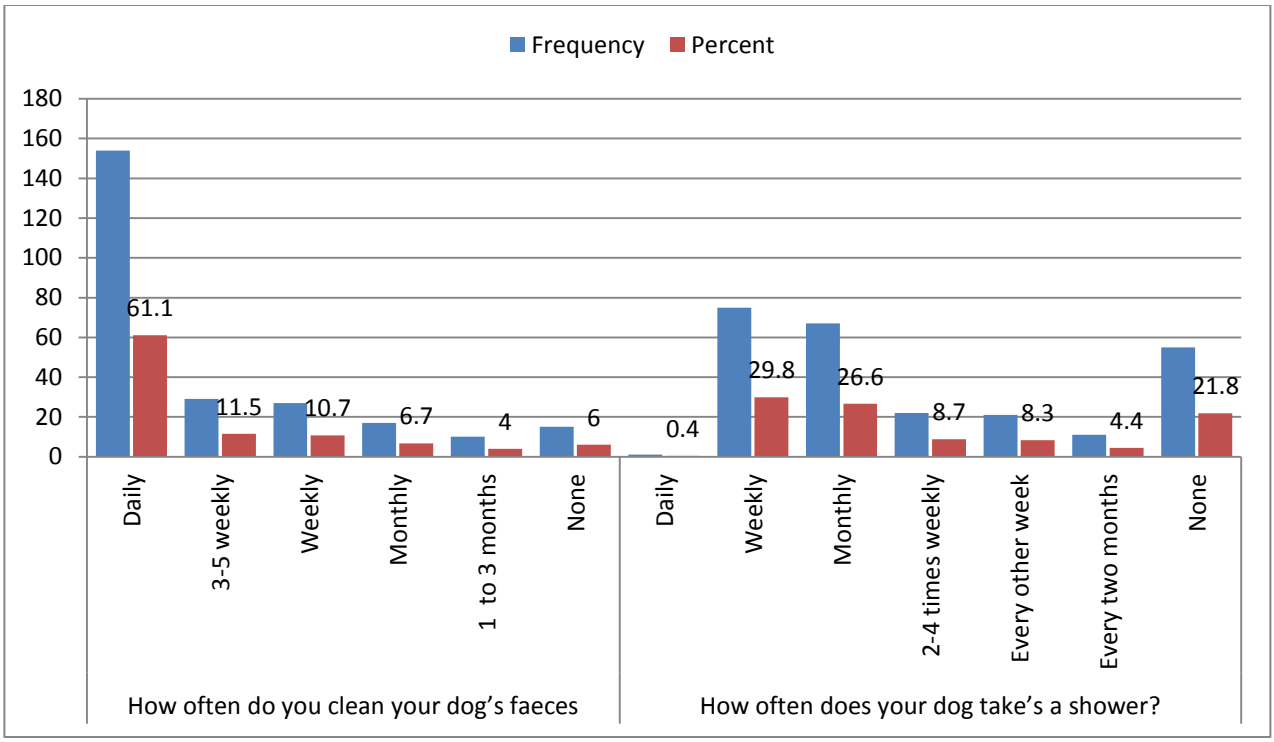

Figure 6: Sanitation practices of dog owners

4.5.3. Zoonotic disease knowledge and educational source of the respondents

Respondents were asked if they were aware of any disease that can be transmitted from dogs to human. Of 252 respondents, 219 **(**87%) of them know a disease that can be transmitted from dogs to human. Of which 95.4% of them knows only rabies and 4.6% of them know both rabies and parasitic diseases as well. The respondents don't know any other disease that can be transmitted from pets to human except rabies and parasitic diseases. Even though the respondents know parasitic disease can be acquired from dogs, none of the respondents name a single zoonotic parasitic disease.

Respondents were also asked whether they received information from any source about diseases that they can get from dogs or precautions to take with dogs to reduce the risk of disease. 61.9% of them have received information regarding zoonotic disease that can be acquired from dogs. Respondents were also asked their source of information and majority (48%) of them who received information about zoonotic diseases obtained this information from their friends, relatives or exposure, only 9% and 9.6% of them received information

from veterinarians and health workers respectively. Very few of them (5.2%) receive the information from media. Responses are shown in Table 12

Table 11: Zoonotic disease knowledge and educational source of respondent

Variables		**Yes N (%)**	**No N (%)**
Do you know a disease that is transmitted from dog to human		219 (87)	33 (13)
If yes what? List them	Rabies	209 (95.4)	
	Rabies and Parasites	10 (4.6)	
Have you ever received information from any source about diseases that you can get from dogs or precautions to take with dogs to reduce the risk of disease?		156 (61.9)	96 (38.1)
If yes, from where?			
Friends and Relatives/Exposure		75 (48)	
Veterinarians		14 (9)	
Health workers		15 (9.6)	
Medias		13 (8.3)	
Internet and Books		6 (3.8)	
School/collage/university		10 (6.4)	
Friends/Relatives +Vets		7 (4.5)	
Friends/Relatives +Health workers		9 (5.7)	
Friends/Relatives +Medias		7 (4.5)	

5. DISCUSSION

This study was the first of its kind in Ethiopia and has greatly improved our knowledge of *Salmonella* in household pet dogs. The epidemiological data gained from this study revealed that the current asymptomatic carriage rate of *Salmonella* in domestic household canines in Addis Ababa was 11.67 %, which is between the lowest and the highest prevalence values of *Salmonella* previously reported from other countries around the world. The prevalence of sub-clinical shedding of *Salmonella* in apparently healthy household dogs has been reported from a number of different countries. Studies have estimated the prevalence of subclinical carriage of *Salmonella* in clinically healthy dogs to be between 0.0% and 43.0% (Carter and Quinn, 2000; Sanchez *et al*., 2002), while other studies reported prevalence of *Salmonella* in faecal samples from clinically healthy or hospitalized dogs to range from 1% to 36% (Greene, 2006). However, review on global *Salmonella* occurrence indicated the decreasing trend of *Salmonella* because of increasing rate of pets consuming commercially processed foods (Greene, 2006).

Overall prevalence of 11.67% in current study was in line with the study conducted in Thailand on 250 apparently healthy dogs which reported 13.2% of *Salmonella* carriage (Arunee *et al*., 2012). Another studies in Iran (Taghi *et al*., 2013), Nigeria (Nwiyi, 2014) and Malaysia (Mustapha *et al*., 2014) were also reported the prevalence of *Salmonella* species to be 13.2%, 12.8% and 9.3% respectively. Similar study done in some developed countries revealed a very low prevalence of *Salmonella* carriage when compared to the present finding, for example, 0.0% in Canada by Murphy *et al*. (2009), 0.23% in UK by Lowden *et al*. (2015), 1% in Turkey by Bagcigil *et al*. (2007), 2.3% in Colorado, USA by Hackett and Lappin (2003) and 3.6% in Trinidad by Seepersadsingh *et al*. (2004). The prevalence of *Salmonella* in dogs is highly variable depending on the immediate environment in which the animals live. For example, *Salmonella* isolation rates from stray dogs have been reported to be significantly higher than those from household dogs (Tsai *et al*., 2007), as these animals presumably survived by scavenging and hunting which increases the risk of consuming *Salmonella* contaminated food (Bagcigil *et al*., 2007).

Salmonella prevalence among dogs appears variable and probably depends on a variety of factors. The differences in the prevalence of *Salmonella* observed between the current study and those previous reports are possibly due to differences in pet husbandry practices, sanitary practice, feeding habit, a high-level public awareness about dog zoonosis and socioeconomic status of dog owners in developed countries for hygiene and make use of the available veterinary cares for their animals (Stull *et al*., 2013). In addition to this, some factors such as geographic location and antibiotic usage are also responsible for the wide variety of *Salmonella* prevalence. The period of study, type of faecal samples, geographical areas, and isolation methods employed in the various studies above may also account for the differences in the prevalence (Seepersadsingh *et al*., 2004).

From the total of 42 *Salmonella* isolates in the present study, 14 different serotypes were identified. *S.* Bronx (16.67%) and *S.* Newport (14.28%) were the dominant serotypes and the higher frequency of these serotypes in this study indicated the serotypes are most prevalent and ubiquitous. Other serotypes such as *S.* Typhimurium, *S.* Saintpaul, *S.* Virchow and *S.* Kentucky were also identified frequently. Some of these serotypes were reported in studies on animals or animal products in Ethiopia: *S.* Newport (Addis *et al*., 2011; Alemu and Zewde, 2012), *S.* Kentucky (Aragaw *et al*., 2007; Zewdu and Poppe, 2009), *S.* Saintpaul (Molla *et al*., 2004; Aragaw *et al*., 2007; Zewdu and Poppe, 2009) and *S.* Typhimurium (Woldemariam *et al*., 2005; Molla *et al*., 2006; Alemu and Zewde, 2012). The occurrences of this serovars in different samples suggest their wide distribution across several animal populations and regions in Ethiopia (Tadesse and Tessema, 2014). The detection of 14 different *Salmonella* serotypes from dogs studied in Addis Ababa suggests that *Salmonella* serotypes are widely distributed and the studied dogs could be important sources of *Salmonella* for humans.

Some of the above serotypes were reported from dogs and other sources from different countries: *Salmonella* serovar Newport were isolated from pet treat in Washington (CDC, 2006) and from beefsteak-patty dog treats that were manufactured in Texas (FDA, 2015b). Inline to our finding, *S.* Typhimurium serotypes were the predominant serotypes reported in dogs by different studies elsewhere. For instance, Glenn *et al*. (1997) reported *S.* Typhimurium from 13 of 23 positive *Salmonella* isolates from asymptomatic dogs in Alaska USA, Ojo and Adeyemi (2009) isolated *S.* Typhimurium serotypes form dogs in Nigeria and

Leonard *et al*. (2011) reported *S.* Typhimurium as the predominant serotype isolated from pet dogs in Ontario, Canada. In agreement to our study, Lefebvre *et al*. (2008) reported *S.* Typhimurium and *S*. Kentucky were the most common serotypes recovered from dogs and Leonard *et al*. (2011) reported *S.* Kentucky was the second most predominant serotype isolated from household dogs in Ontario, Canada.

To our knowledge, S. Bronx, S. Chailey, *S.* Indiana, *S.* Minnesota and *S*. Tarshyne were reported for the first time in Ethiopia. In addition, very few reports have showed the isolation of *S*. Bronx, *S.* Chailey and *S*. Tarshyne form dogs worldwide. Most of them were isolated from other sources. For example, *S*. Chailey was reported in Australia from eggs (Vicki *et al*., 2009), from human patients in Korea (Shukho *et al*., 2010), from hens and eggs associated with food-borne infections in Japan (Otomo *et al*., 2007) and from Human patients in New Zeland (New Zealand Public Health Surveillance, 2012). *S*. Tarshyne serotypes were identified from antelope, ostrich and caracal (Münch *et al*., 2012). Similar to our finding, *S*. Indiana and *S*. Minnesota were isolated form clinically healthy dogs in UK (Carter and Quinn, 2000; Philbey *et al*., 2013) and in USA (Carter and Quinn, 2000).

Many human illness outbreaks associated with most of the above serotypes were reported from different countries around the world. For instance, *S*. Typhimurium, *S*. Newport, *S*. Saintpaul and *S*. Braenderup were causes of outbreaks that occurred between 2009 and 2014 in the USA (CDC, 2014b). Similar outbreaks due to *S*. Anatum in Japan (Ebuchi *et al*., 2006), *S*. Muenchen in Germany (Buchholz *et al*., 2005), *S*. Kentucky in USA (Lu *et al*., 2013), *S*. Indiana and *S*. Muenche in USA (CDC, 2015c) were recorded. Outbreaks and human illnesses duet to *S*. Newport (CDC, 2012b), *S*. Braenderup (CDC, 2014a), *S*. Typhimurium (CDC, 2012b), *S*. Indiana (CDC, 2015a), *S*. Muenchen (CDC, 2015a), *S*. Anatum (Krause, 2007) and *S*. Saintpaul (FDA, 2014) were reported in multiple states in USA. *Salmonella* Virchow outbreak in Switzerland (Mario *et al*., 2011), in New Delhi (Randhawa *et al*., 2006), *S*. Typhimurium outbreak in Australia (Vicki *et al*., 2009), *Salmonella* Newport outbreak in pilgrims during Hajj (Olaitan *et al*., 2015) were also among the NTS outbreaks reported worldwide. *S*. Typhimurim and *S*. Newport were among the big five *Salmonella* serotypes causing foodborne illness outbreaks according to food safety news (Robinson, 2013).

Statistically significant association found between *Salmonella* carriage and the presence of Diarrhea (loose stools) in the past two months in the current study was presumably due to *Salmonella* being one of the causes of clinical disease manifested by diarrhea in these dog populations. Dogs generally seem to be resistant to *Salmonella* infection and most cases are latent and non-clinical (Greene, 2006). However, clinical cases of salmonellosis have been reported in dogs which in severe cases can result in diarrhea, vomiting, fever, depression, abortion and death (Merck, 2015).

Ingestion of contaminated food is thought to be the predominant risk factor for *Salmonella* infection and *Salmonella* has been isolated at high frequency from raw dog food and asymptomatic carriers developed after experimental oral inoculation, with shedding observed for several weeks (Finley *et al*., 2007). Raw food (i.e., composed of raw meat and bones) are becoming increasingly popular in pet dog populations and have been associated with the carriage of *Salmonella* in dogs, with several studies have investigated the contamination of these diets and their ingredients with *Salmonella* and other bacteria (Weese *et al*., 2005; Finley *et al*., 2008). Most (59%) of the respondents in our study reported that they fed raw meat to their dogs and majority (79%) of them obtained the meat from local open market which is more likely to be contaminated. Contrary to this, a study in Canada, Ontario by Stull *et al*. (2013) reported only 28% of the dog owners fed high-risk foods (i.e. raw eggs, raw meat, or raw animal product treats) to their dogs. This is mainly due difference in the economic status of the dog owners and in countries like Ethiopia, even if there are some dog owners who can afford commercial pet food, it is not readily available on market.

In this study statistically significant association is observed between drinking contaminated water (Rain water and water from toilet bowls) and *Salmonella* carriage. Dogs which drink rain water and water from toilet bowl showed high infection rate than those who drinks only clean municipal water with odds ratio of 5.1 and 4.08 respectively. This is due to the fact that as *Salmonella* is ubiquitous in nature and chance of contamination of the water in the toilet and rain water with *Salmonella* positive human or animal feces is high. Animal and human feces are the most common source of *Salmonella* (CDC, 2015; Hoelzer *et al*., 2011).

The antimicrobial sensitivity test results of this study revealed that 42.86% of the isolates were resistant to at least one antimicrobial drug and the prevalence of resistance was highest to ampicillin (26.20%), amoxicillin-Clavulanic acid (23.80%), doxycycline (23.80%) and oxytetracycline (23.80%). Previous antimicrobial resistance studies of canine *Salmonella* have reported higher levels of resistance than reported here, and also commonly found resistance to tetracycline and streptomycin (Seepersadsingh *et al*., 2004; Tsai *et al*., 2007). A study conducted in Nigeria (Ojo and Adeyemi, 2009) in diarrheic and non-diarrheic dogs demonstrated a high resistance to ampicillin (47.1%), amoxicillin-clavulanic acid (52.9%), streptomycin (35.3%), sulfamethoxazole-trimethoprim (76.5%) and gentamicin (35.3%), which is much higher than the present study. While Only 26.2%, 23.8%, 14.28% 9.52% and 2.38% of the isolates were resistant to the corresponding antimicrobials, respectively, in our study. Another study in Malaysia reported that 66.7% of *Salmonella* isolates isolated from dogs were resistant to at least one antimicrobial drug and none of the isolates were resistant to gentamycin and amoxicillin-clavulanic acid (Mustapha *et al*., 2014). In contrast to our result, a study on household and stray dogs in northern Taiwan (Tsai *et al*., 2007) reported relatively higher rate of resistance to nalidixic acid (51.5%), gentamicin (33.3%) and ciprofloxacin (18.2%).

Thirty eight percent of the *Salmonella* isolates belonging to *S.* Newport, *S.* Haifa, *S.* Anatum, , *S.* Kentucky, *S.* Saintpaul, *S.* Chailey and *S.* Indiana were multiple antimicrobial resistant (to two or more drugs). All *Salmonella* Newport serotypes were MDR to three and more antimicrobials and all *S.* Typhimurium serotypes (except one which is resistant to Doxycycline) were Pansusceptible to all antimicrobials tested. All *S.* Bronx serotypes were pansuceptible to all antimicrobials, except one which is MDR to cephalothin, cefoxitin, ampicillin and gentamicin. Two *S.* Saintpaul, One *S.* Haifa and one *S.* Indiana serotypes were MDR to five and more antimicrobials tested. None of the *S.* Virchow, *S.* Muenchen, *S.* Braenderup and *S.* Minnesota isolates were resistant to antimicrobials tested and high percent of susceptibility was exhibited in the isolates to nalidixic acid, gentamicin, kanamycin, ceftriaxone and chloramphenicol. It was found that all of 42 (100%) isolates were susceptible to ciprofloxacin. This study is in agreement with Ojo and Adeyemi, (2009); Tsai *et al*., (2007) and Nwiyi, (2014), which reported 100% susceptibility to ciprofloxacin in *Salmonella* isolates from dogs. This result was comparable with previous reports by Molla *et al*. (2006) from

central part of Ethiopia among isolates of sheep and goat meat, Akinyemia *et al*. (2005) from Nigeria, from human isolates and Addis *et al*., (2011), among isolates of *Salmonella* from dairy farms in Addis Ababa.

All of the isolates were susceptible to Amikacin. The result was in agreement with previous reports by Molla *et al*. (2003) on *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Eguale *et al*. (2015) from human isolates among diarrheaic patients in Addis Ababa. Other study in Canada on *Salmonella* isolates recovered from pet dogs, reported that amikacin is 100% effective against the isolates (Leonard *et al*., 2011). Amikacin and ciprofloxacin could be useful in the treatment of the majority of cases of salmonellosis as shown by their effectiveness against a high percentage of the *Salmonella* isolates in this study.

Antimicrobial resistant tests in *Salmonella* isolates from other animals at different part of Ethiopia showed relatively higher resistance rate as compared to this study. The reason behind this discrepancy could be differences in serotype composition reported by the studies, differences in the antibiotics commonly used and the habits of pet owners regarding pet husbandry. In developing countries like Ethiopia, it is not a common practice to take dogs to vet clinics (Gebretsadik *et al*., 2014; Dejene *et al*., 2013). Similarly, as it is seen in this study, majority (70%) of dog owners in Addis Ababa didn't take their dogs to vet clinics (at least once a year) for treatment and majority of them don't give a drug for their dogs. This could be the reason for the relatively low antimicrobial resistance rate seen in this study. However, the widespread use of antibiotics in food animals resulted in the selection of resistance to *Salmonella* in their intestine, which subsequently entered the food chain or in other ways reached humans and other animals (Phillips *et al*., 2004). Since antibiotic resistance has an important health, social and economic impact, there is a need for stronger scientific and public health efforts to better regulate, control and monitor the use and abuse of antimicrobials (FDA, 2015a)

Investigation of dog owner's knowledge, attitude and practice related to pet husbandry, pet contact, sanitation practice and zoonotic disease knowledge was done in this study. The results revealed that 70.2% of respondents don't take their dog to veterinary service (at least once a year) and 85.7% of them don't give any medicine for their dogs. This result indicates

that most of dog owners in Addis Ababa give a little care for their dogs and they don't even think that dogs need medical treatment when they became ill; Most of them think that vet clinics give services only for antirabis vaccination rather than treating sick pets. The overwhelming majority (84.1%) of dog owning households reported they touched the dogs on regular basis. The nature of the contact was very close in many households where 51.6% of them would have either direct or indirect mouth contact with their dogs. These estimates are similar to a previous study in Netherlands in which dogs were permitted to lick their owners' face (50%) and 18%, reportedly slept in an adult's bed (Overgaauw *et al*., 2009). In our study 27% of the respondent dog owners allowed their dogs to sleep in living room. The disease risk associated with allowing pets such as dogs and cats to sleep in living rooms is unquantified and presumably dependent on many factors, including the level of contact between the pet and family member and level of disease-risk posed by the pet due to husbandry practices. However, sleeping with pets has been identified as a risk factor for several diseases, prompting some to discourage this practice by higher risk individuals (Chomel and Sun, 2011)

In this study, the reported hand washing was high (78% of respondents reported they washed hands sometimes or greater after touching the pet, its feces, or housing). Of which 34.13% of them washed always, 35.32% usually and 30.53% sometimes and about 1/5 of the respondents never wash their hands after having contact with their dog. In contrast to our study most of dog owners in developed countries wash their hands less frequently after having contact with their dogs. For example, Overgaauw *et al*. (2009) reported that 50% of dog owners in Netherland washed their hand after having contact with their dogs and Westgarth *et al*. (2008) reported that only 15% of dog owners washed their hand after touching the dog in Cheshire, England. The reason for this difference could be as most dog owners in developed country give much attention for their dogs regarding feeding, sanitation and health, they don't wash their hand all the time after having contact because they believed that their pets are healthy. The level of hand hygiene documented in our study is likely adequate for those households which cares about the hygienic status of their dogs; however, for owners who are not concerned about hygienic status of their dog, hand washing should always be practiced (Stull *et al*., 2013).

Eighty-seven percent of the respondents in the current study know zoonotic disease that can be transmitted from dogs to human and most of them (95.5%) knows only rabies and only 4.4% of them know parasitic diseases beside to rabies. The knowledge they have about rabies was not full and comprehensive, as some of the respondents believed that rabies can be transmitted by air and can be treated with traditional medicines. The respondents don't know any other disease that can be transmitted from pets to human except rabies and parasitic disease. Inline to this finding, a study in Hawassa (Dejene *et al*., 2013) showed that 85.7% of respondents have awareness about zoonotic canine diseases. However, their awareness was mainly restricted to rabies which accounted 96.96% and only few of them have awareness about canine zoonotic parasites which only holds 3.0%. In another study in Ambo (Zewdu *et al*., 2010), only 44.3% of the owners have awareness about the role of dogs in transmitting diseases to human. The awareness was only for rabies; none of them had awareness of other canine zoonotic diseases. None of the respondents in our study know *Salmonella* is zoonotic disease that can be acquired from dogs. As awareness of zoonotic disease risk is a prerequisite for effective prevention, the limited zoonotic disease knowledge of the public is a concern.

The low zoonotic disease awareness observed by respondents is not surprising as only 61.9% of respondents reported having ever received information about pet-associated diseases or precautions to reduce the risk of these diseases. The majority (48%) of respondents who have awareness about canine zoonotic diseases obtained this information from their friends or relatives. Only 9% and 9.6% of them received information from veterinarians and health workers respectively. By contrast Bingham *et al*., (2010) in USA and Palmer *et al*. (2010) in Australia reported that Veterinarians and the Internet were most frequently reported sources as providing this information to pet and non-pet owners, respectively. A study in New York (Gursimrat and Devinder, 2014) reported that 40% of participants reported their veterinarian as their primary source of information, while 20% and 5% participants reported internet and media were their source of information respectively. The limited involvement of physicians and public health was not surprising. Several studies have indicated that physicians often rely on veterinarians for advising the public about the potential for zoonotic disease and thus discuss this topic with their patients less frequently than veterinarians (Katagiri and Oliveira, 2008; Gursimrat and Devinder, 2014). However, in our study the proportion of individuals who received information from Veterinarians was very low (9%) which is contrary to other

studies conducted in other developed countries. In order to minimize zoonotic diseases that can be acquired from pets and other animals, veterinarians, public health workers, schools and medias should play a major role in creating awareness about these diseases. The government should also create a policy which enables the above stakeholders to strongly teach and inform the community about zoonotic diseases.

6. CONCLUSION AND RECOMMENDATIONS

The result of this study revealed high prevalence of asymptomatic carriage of different *Salmonella* serotypes in dogs and moderate resistant to antimicrobials used both in human and veterinary practices. Among the antimicrobials tested, the highest level of resistance was observed against ampicillin, amoxicillin+clavulanic acid, doxycycline and oxytetracycline. A total of fourteen different *Salmonella* serotypes were isolated with *S*. Bronx, *S*. Newport, *S*. Indiana, *S*. Kentucky, *S*. Saintpaul and *S*. Virchow being the most dominant. *S*. Bronx, *S*. Chailey, *S*. Indiana, *S*. Minnesota and *S*. Tarshyne were isolated for the first time in Ethiopia. Detection of serotypes commonly causing salmonellosis in human patients in Ethiopia and elsewhere such as *S*. Typhimurium, *S*. Kentucky, *S*. Virchow, *S*. Newport poses a major threat to public. The presence of diarrhea in the past months, cleaning frequency of dog's faeces, sleeping place of the dog and drinking contaminated waters were found to be the risk factors for *Salmonella* infection.

KAP assessment on pet-related management, contact-related attitude and zoonotic disease knowledge, revealed that majority of dog owners has insufficient knowledge regarding pet feeding, husbandry, sanitation and zoonotic diseases that can be acquired from dogs. So creating awareness is the best way for prevention of any zoonotic diseases that can be acquired from dogs. Based on the above conclusion, the following recommendations are forwarded:

- Similar study should be conducted in other provinces and areas to determine regional differences and similarities on prevalence and serotype distribution of *Salmonella*, thereby increasing the utility of future pet ownership guidelines.
- More detailed research investigating the role of antimicrobial use and AMR development in healthy dogs needs to be conducted, as well as comparative testing of dogs and humans in the same households, in order to determine common risk factors and sources of exposure
- Raw meat and other raw animal products should not be fed to dogs within households or in contact with vulnerable people

- Buy fresh pet foods (meats) from licensed butchers when possible and prohibit access to non-potable water, such as surface water or toilet bowls
- Keep dogs confined when possible; walk on leash to prevent hunting, coprophagia, and garbage eating
- Seek veterinary care at first sign of illness manifested with diarrhea
- Creating community awareness on pet husbandry, zoonotic canine diseases, prevention measures and good hygienic practices
- Public health professionals, Vets and different Medias should be actively involved in awareness creation.

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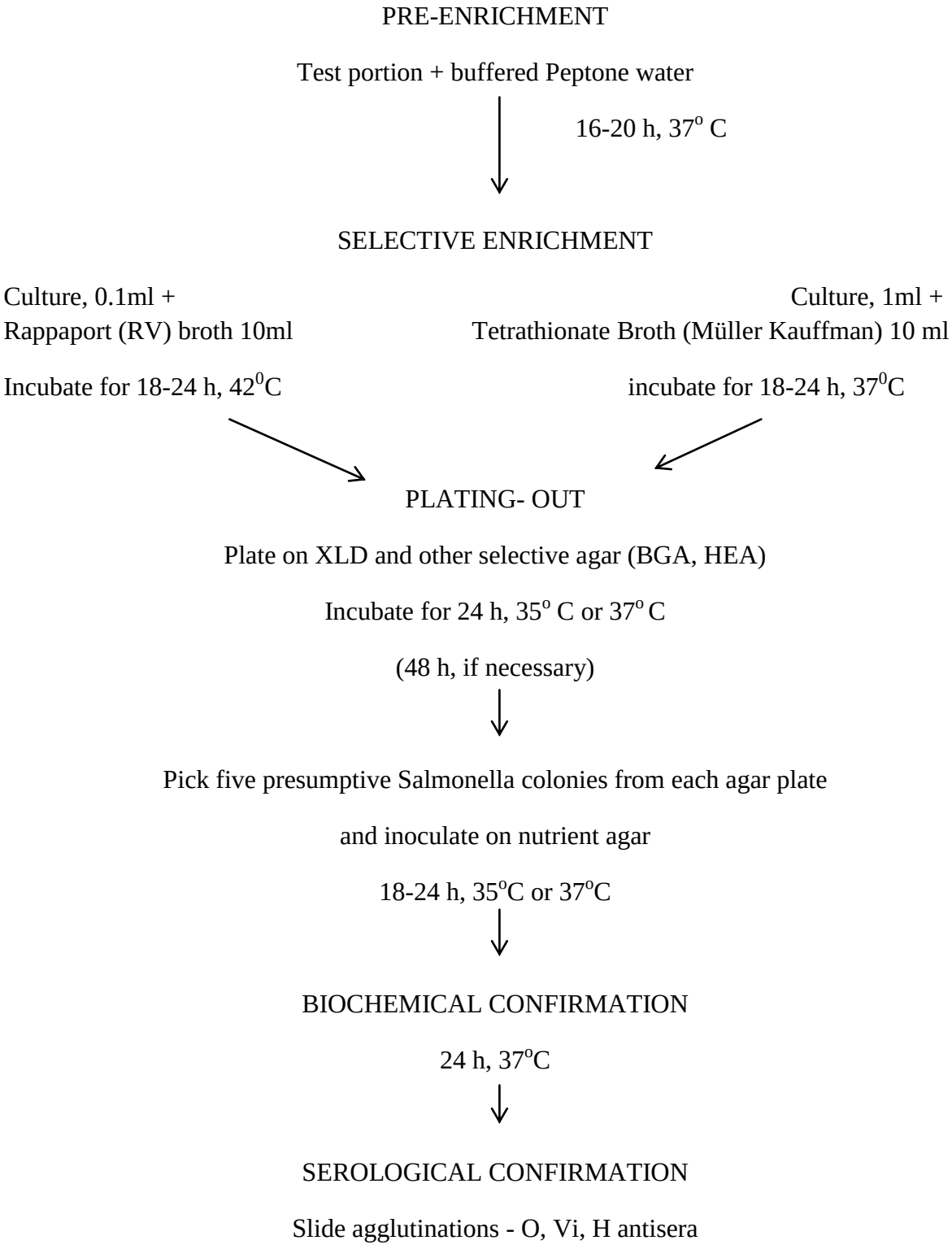
8. APPENDICES

Annex 1. ISO Salmonella isolation procedure



Annex 2. Laboratory results record sheet format

Sample Number	Colony Characteristics on			Biochemical Tests				PCR result
	XLD	BGA	HEA	TSI	Urease	Lysine	Citrate	
1								
2								
3								
4								
360								

Annex 3. Questionnaire Format for Pet Owners

1. Name of the owner _____Kifle Ketema _____
2. Dog Name_____ Age _____Sex? Male [] Female []
3. Neutered? Yes [] No [] Unsure []
4. Breed? Local [] Exotic [] Cross []
5. For what purpose do you own (use) the dog?
   Guard, [] hobby [] both guard & hobby [] Other_____
6. Where does your dog spend the majority of its time?
   All the time in the house [] Mostly in the house [] Half inside []
   Mostly outside [] All the time outside []
7. Where does your dog **most commonly** defecate? _____
8. Diarrhea in the last 60 days? Yes [] No []
9. What does your dog eats? Meat [] Raw egg [] table scraps (Human Foods) []
   Raw milk [] commercial foods []
10. Where did you obtain the meat? Licensed butcher [] Not licensed butcher []
    Supermarket [] other, please specify_____
11. What type of meat did you feed? Fed raw [] Fed after cooking []
    a. Any other food that your dog eats? _____
12. Where the dog does_sleeps? Living room[] Dog's house [] Bedroom [] outside the house [] outside the compound []
13. Is the dog permitted to sit and/or sleep on furniture? Yes [] NO []
14. Does your dog ever catch?

Birds	Rodents (mice-Rat)	Reptiles(lizard-snakes)	Amphibians(frogs)	Insects	Rabbits

15. Did the dog see a veterinarian on a regular basis (at least once a year)? Yes No
16. Did you give the dog any medicine for diseases? Yes -------No----------
    a. When? _____ If so what? _____

17. How often do you clean your dog's Faeces? Daily [] 3-5 times a week [] Weekly [] Monthly [] one month to three months [] Never_____

18. If you pick-up the faeces/droppings or empty the litter tray, what do you do with them? ☐Placed in a waste bin, ☐Put on or buried in the garden, ☐Put in compost heap, ☐compost tumbler and ☐Not applicable. Who clean it_____

19. How often does your dog take's a shower? Daily [] Weekly [] 2 to 4 times weekly [] every other week [] Monthly [] every two months [] None/above three months []

20. Is it common for you and your family members to wash your hands after having contact with the dogs? Yes [] NO []

How often? Always [ ] Usually [ ] Sometimes [ ] Never [ ]

21. What is the main source of drinking water for the dog? Municipal city water [] Well [] used water (after washing hand etc.)_____ other_____

22. In addition to the main drinking water sources, does your dog ever drink from these sources of water? Rain water Yes [] No [] Toilet Yes [] No [] other_____

23. For owners and all children

	Yes	No
Having mouth contact with any part of the dog		
Touching the dog		
Touching and playing with the animals poop		

24. Do any of the children (member of your family) play in the same area as where the dog(s) go to the bathroom? Yes [] No []

25. Do you know a disease that is transmitted from dog to human Yes [] No []

If yes what? List them_____

How diseases can transmit from dogs to human? _____

26. Have you ever received information from any source about diseases that you can get from dogs or precautions to take with dogs to reduce the risk of disease? Yes [] No []

27. If yes, from where? Health workers [ ] Veterinarian [ ] Friends/relatives [ ] Internet [ ] Books [ ] Television/newspaper [ ] other_____

This completes the questionnaire. Thank you, I really appreciate your participation in this important study.

Annex 4. Composition and preparation of culture media and reagents

A. Buffered Peptone Water (DIFCO ™, BD USA)

Approximate Formula* Per Liter

Approximate Formula	gm/lit
Peptone	10
Sodium Chloride	5
Disodium Phosphate	3.5
Monopotassium Phosphate	1.5

Directions: - Dissolve 20.0 g of powder in 1 L of purified water. Mix thoroughly. 2. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures.

B. Tryptic Soy Broth (TSB) (Soyabean-Casein Digest Medium) (DIFCO, BD USA)

Approximate Formula* Per Liter

Formula	Gm/lit
Bacto™Tryptone (Pancreatic Digest of Casein)	17
Bacto Soytone (Peptic Digest of Soybean Meal)	3
Glucose (=Dextrose)	2.5
Sodium Chloride	5
Dipotassium Hydrogen Phosphate	2.5
pH 7.3 ± 0.2	

Directions: - Suspend 30.0 gm. of the powder in 1 Litre of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder. Autoclave ate 121^0c for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures.

C. Xylose Lysine desoxycholate Agar (XLD) (Oxoid, England)

Composition (g/Litre):

Typical Formula*	**gm/litre**
Yeast extract	3
L-Lysine HCl	5
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1
Sodium chloride	5
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 ± 0.2 @ 25°C	

Directions: Suspend 53g in 1 liter of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVERHEAT. Transfer immediately to a water bath at 50°C. Pour into sterile Petri dishes as soon as the medium has cooled.

D. Hekton Enteric Agar (Oxoid, England)

Composition (g/Litre):

Typical Formula*	**gm/litre**
Proteose peptone	12
Yeast extract	3
Lactose	12
Sucrose	12
Salicin.	2
Bile salts No.3	9
Sodium chloride	5
Sodium thiosulphate	5
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue.	0.065
Agar	14
pH 7.5 + 0.2 @ 25°C	

Directions

Suspend 76g of the medium in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. DO NOT AUTOCLAVE. Cool to 50°C and pour plates.

E. Brilliant Green Agar (Difco, USA)

Approximate Formula* Per Liter

Formula	Gm/lit
Proteose Peptone No. 3	10
Yeast Extract	3
Lactose	10
Saccharose.	10
Sodium Chloride	5
Agar	20
Brilliant Green	12.5
Phenol Red	0.08

Directions for Preparation: Suspend 58 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Auto clave at 121° C for 15 minutes. Cool to 50°C and pour plates.

F. Mueller-Hinton Agar (OXOID, ENGLAND)

Composition (g/Litre):

Typical Formula*	**gm/litre**
Beef, dehydrated infusion from	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17
pH 7.3 ± 0.1 @ 25°C	

Directions

Add 38g to 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour plates

G. **Nutrient Agar Medium (OXOID, ENGLAND)**

Typical Formula*	gm/litre
Yeast extract	4
Tryptone	5
Glucose	50
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Agar	15
pH 5.5 ± 0.2	

Directions: Suspend 75g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. If required the pH may be adjusted to 6.5 by the addition of 1% sodium bicarbonate solution

H. Tryptone Soya Agar (Casein soya bean digest agar) (OXOID, ENGLAND)

Composition (g/Litre):

Formula	gm/litre
Pancreatic digest of casein	15
Enzymatic* digest of soya bean	5
Sodium chloride	5
Agar	15
pH 7.3 ± 0.2 @ 25°C	

Directions: Add 40g to 1 litre of distilled water (purified as required). Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

I. Muller-Kauffmann Tetrathionate Broth Base (OXOID, ENGLAND)

Composition (g/Litre):

Typical Formula*	**gm/litre**
Tryptone	7
Soya peptone	2.3
Sodium chloride	2.3
Calcium carbonate	25
Sodium thiosulphate	40.7
Ox bile	4.75
pH 8.0 ± 0.2 @ 25°C	

Directions:-Suspend 82g in 1 litre of distilled water and bring to the boil. Cool below 45°C and add, just prior to use, 19ml of iodine solution and 9.5ml of a 0.1% brilliant green solution. Mix well and fill out into sterile tubes or flasks

J. Rappaport-Vassiliadis Soya Peptone Broth (RVS BROTH) (OXOID, ENGLAND)

Composition (g/Litre):

Typical Formula*	**gm/litre**
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Di-potassium hydrogen phosphate	0.18
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036

Directions:-Suspend 26.75g in 1 litre of distilled water and heat gently to dissolve. Dispense 10ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

K. Nutrient Broth (OXOID, ENGLAND)

Composition (g/Litre):

Typical Formula*	**gm/litre**
`Lab-Lemco' powder	1
Yeast extract	2
Peptone	5
Sodium chloride	5
pH 7.4 □ 0.2 @ 25°C	

Directions: Add 13g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

L. Urea Agar Base (Christensen) (Autoclavable) (Himedia, India)

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	1
Dextrose	1
Sodium chloride	5
Disodium phosphate	1.2
Monopotassium phosphate	0.8
Phenol red	0.012
Agar 1	15
Final pH (at 25°C) 6.8±0.2	

Directions: - Suspend 24.01 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well. Dispense into sterile tubes and allow to set in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily

M. Triple Sugar Iron Agar (TSI) (Difco, USA)

Approximate Formula	Gm/lit
Beef Extract	3
Yeast Extract	3
Pancreatic Digest of Casein	15
Proteose Peptone No. 3	5
Dextrose	1
Lactose	10
Sucrose	10
Ferrous Sulfate	0.2
Sodium Chloride	5
Sodium Thiosulfate	0.3
Agar	12
Phenol Red	24

Directions: Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into tubes and autoclave at 121°C for 15 minutes. Cool in a slanted position so that deep butts are formed.

N. Simmons Citrate Agar (Himedia, India)

Composition

Ingredients	Gms / Litre
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Sodium citrate	2
Sodium chloride	5
Bromothymol blue	0.08
Agar	15
Final pH (at 25°C) 6.8±0.2	

Directions: - Suspend 24.28 grams in 1000 ml distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distributein tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Precaution: Before using water, ensure pH of water is 6.5 to 7.0.Initial color of the medium may deviate from expected Color, if the above precaution is ignored.

O. Lysine Iron Agar (Micro Master Lab, India)

Formula/liter

Ingridents	Gms/liter
peptic digest of animal tissue	5
yeasat extract	3
dextrose	1
L-Lysine	10
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
bromocresol puple agar	0.02
Agar	15
final ph:(at 25^0c) 6.7 ±0.2	

Directions: - Suspend 33 grams of the medium in one liter of distilled water. Heat if necessary, to dissolve the medium completely. Distribute into tubes as desired**.** Autoclave ate 121^oc, 15 psi pressure, for15 minutes/validated cycle. Cool the tubes in slanted position to from slants with deep butts

P. Accupower Taq PCR PreMix (Bioneer, Korea)

The Accupower Taq PCR PreMix is a convenient lyophilized PCR master mix containing Taq DNA polymerase, dNTPs, reaction buffer, tracking dye, and stabilizer and is aliquoted in 8-strip PCR tubes. The premix retains its activity for over six month at room temperature and is stable for two years in -20ºC freezer. Accupower Taq PCR PreMix is available with or without tracking dye, depending on your application. If purchased with tracking dye, reactions can be loaded on agarose gels without adding loading buffer.

Contents

Reaction Volume/ Component	**20µl**	**50 µl**
Taq DNA polymerase	1 U	2.5 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 µM	Each 250 µM
Reaction buffer, (with 1.5 mM MgCl2)	1 X	1 X
Stabilizer and tracking dye[1)]	Trace	Trace

1) Accupower Taq PCR PreMix is premixed with Xylene Cyanol. Xylene Cyanol migrates at approximately 4kb on 1% agarose gel

Protocol

1. Thaw template DNA and primers before use.

2. Add template DNA and primers into the Accupower Taq PCR PreMix tubes.

3. Add distilled water into the Accupower Taq PCR MasterMix tubes to a total of 20µl (K-2601, K-2602) or 50µl (K-2603, K-2604). Do not calculate any volume for the dried pellet.

4. Dissolve the lyophilized blue pellet completely and spin down either by using Bioneer's Vortex/Centrifuge or by pipetting up and down several times and then briefly spinning down

5. Perform the reaction under the following conditions: predenaturation 95ºC (1 min), denaturation 95ºC (30 sec), Annealing 45-65ºC (30 sec), Extension 72ºC (30 sec-1min/kb), Final extension 72ºC (optional, 3-5min)

7. Maintain the reaction at 4°C after the completion of amplification. It is recommended to store the sample at -20°C until use.

8. Load the reaction mixture directly on agarose gel to analyze the PCR products. If purchased with tracking dye, the reaction can be directly loaded onto the gel.

Q. Remel McFarland Equivalence Turbidity Standard visual comparison card

Remel McFarland Equivalence Turbidity Standards are used as standards in adjusting densities of bacterial suspensions.

Original McFarland standards were prepared by adding $BaCl_2$ to H_2SO_4, resulting in BaSO4precipitation.The McFarland Equivalence Turbidity Standards are prepared from suspensions of uniform polystyrene microparticles with absorbance values similar to the original BaSO4standards. Stability of suspensions, shelf life, and ease of comparison has been improved with the McFarland Equivalence Turbidity Standards.

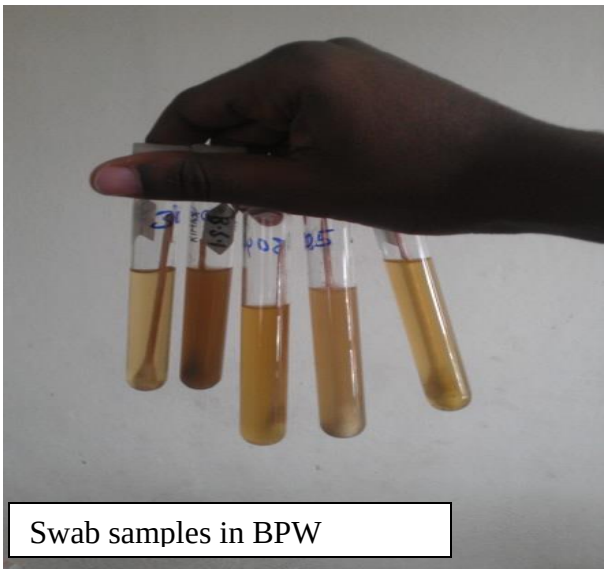
PRINCIPLE

Polystyrene microparticles are suspended in a special buffer and adjusted to an acceptable absorbance range using a spectrophotometer with a 1 cm light path set at 600 nm or 625 nm, depending on the standard used. Adjusting a bacterial suspension turbidity to the McFarland Equivalence Turbidity Standard produces bacterial counts in an expected range.

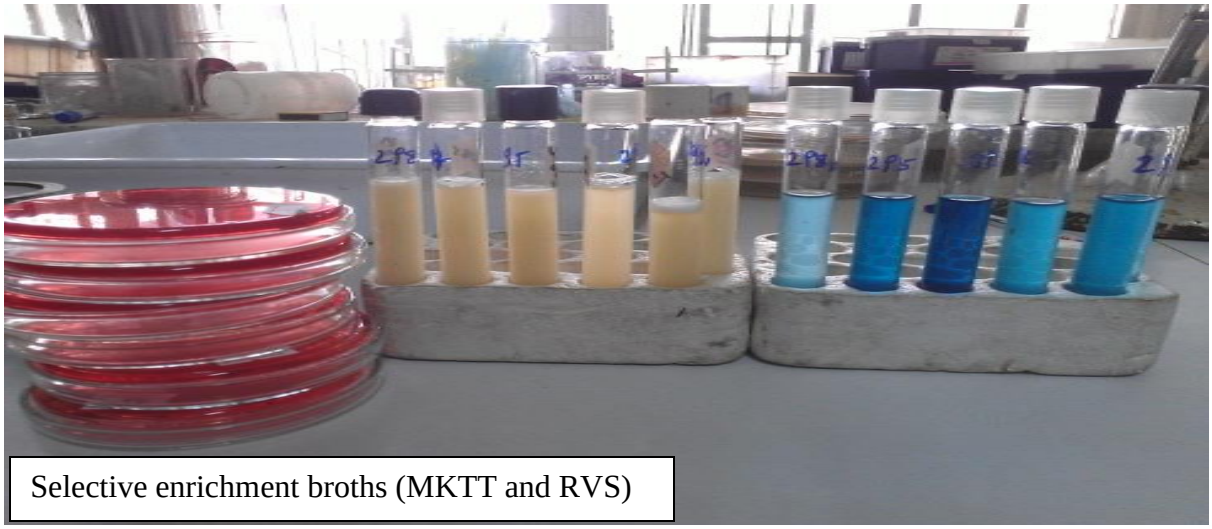
Annex 5. Pictures taken during lab procedures

Sample collection sterile cotton swab

Rectal faecal swab collection

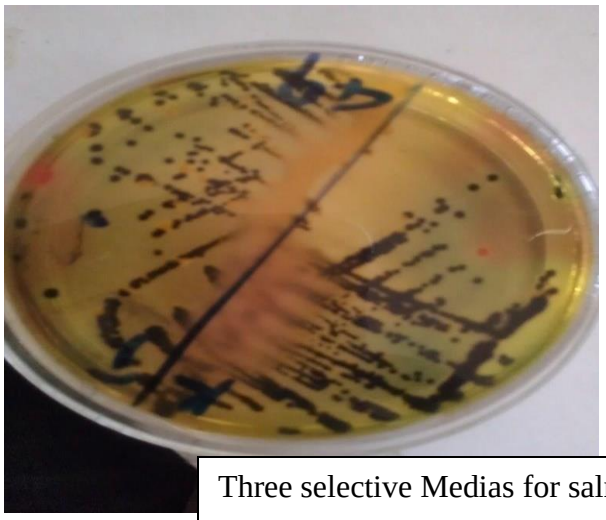


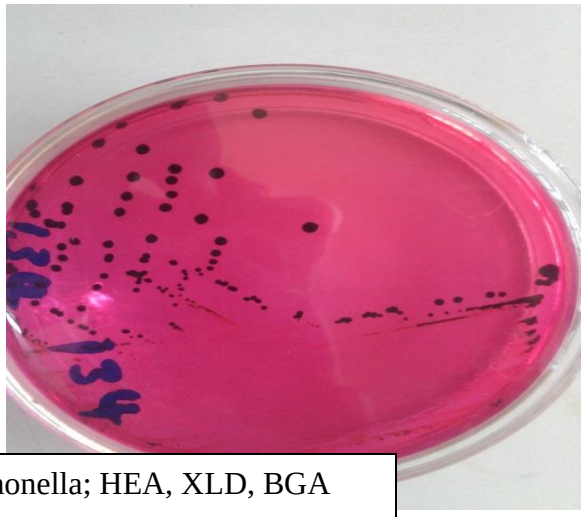
Swab samples in BPW



Selective enrichment broths (MKTT and RVS)

Culturing and transferring pre enrichment broths to selective enrichment broths



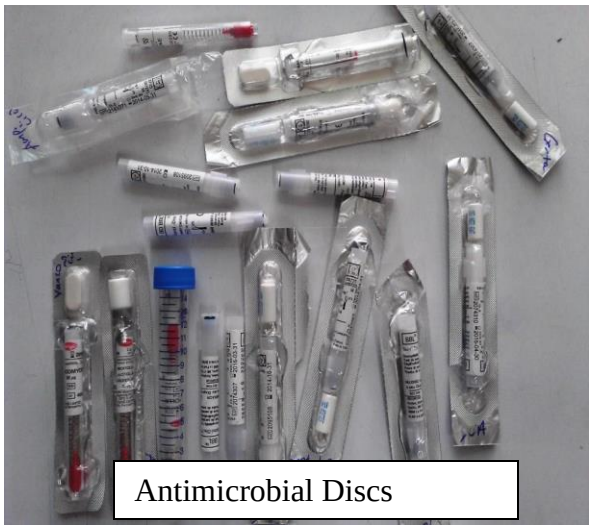


Three selective Medias for salmonella; HEA, XLD, BGA

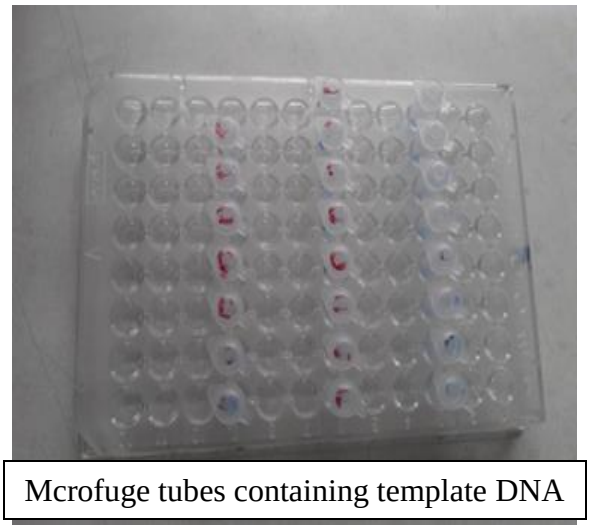
Swabbing bacterial suspension on MHA

Biochemical test

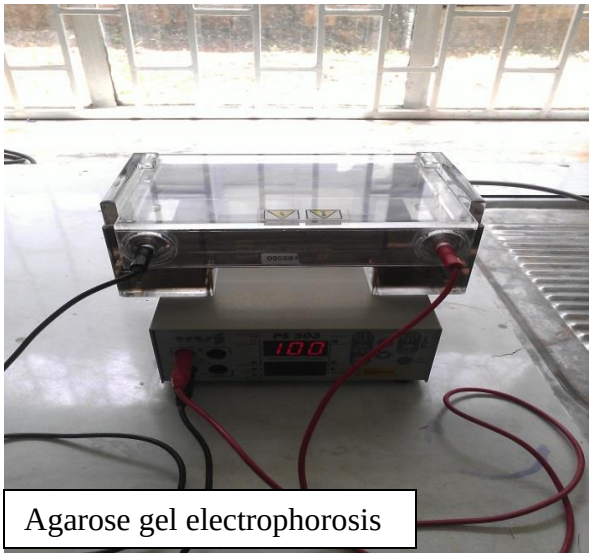
Placing antibiotic discs on MHA



Antimicrobial Discs

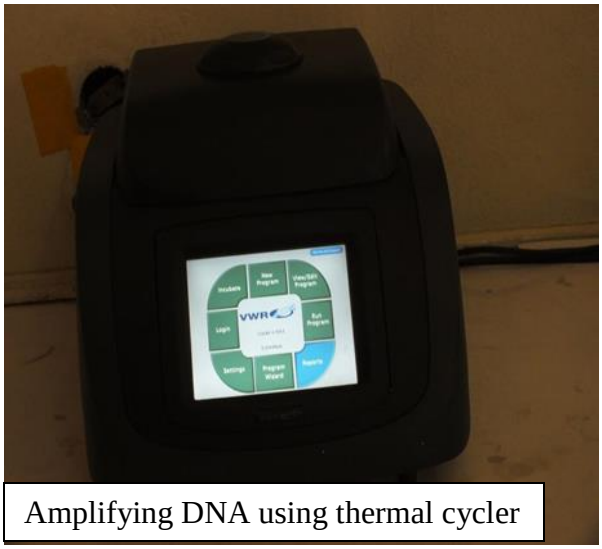


Mcrofuge tubes containing template DNA



Agarose gel electrophorosis

Adding template DNA to NFW filled PCR tubes



Amplifying DNA using thermal cycler

Gel documentation