Sudan University of Science and Technology

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Detection of *Salmonella* species in Slaughtered Cattle, Raw Meat and Meat products in Khartoum State

الكشف عن انواع السالمونيلا في الابقار المذبوحه واللحوم الطازجة و منتجات اللحوم في ولاية الخرطوم

A thesis Submitted in Fulfillment of the Requirements Degree of Doctor of Philosophy (Ph.D) in Veterinary Medicine

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Dedication

To my father’s soul

To my mother, sisters and brothers

To my husband

To my sons and daughters

Acknowledgement

I would like to thank Allah for giving me the Knowledge and supported me to complete this research.

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List of Abbreviations

RTE	Ready to eat Meat
PCR	Poly chain reaction
DCA	Deoxy cholate agar
DNA	Deoxyribo nucleic Acid
IOFS	International Organization for Standardization
CFU	Colony Forming Unit
SPSS	Statistical Package for the Social Sciences
MR	Methyl red
VP	Vogues- proskauer reaction
LPS	Lipopoly saccharide
WHO	World health organization
NVI	National Veterinary Institute
RASFF	Rapid Alert System for Food and Feed
CDC	Centers for Disease Control and Prevention
mPCR	MultiplexPCR
H2S	Hydrogen sulphide

Abstract

The purpose of this study was to detect degree of *Salmonella* spp. Contamination from cattle slaughtered, raw cattle meat and ready to eat meat RTM products in Khartoum State – Sudan, quantitatively by counting the total viable, and qualitatively by the isolation and identification of *Salmonella spp* using conventional methods (ISO 11290-1, 2004), biochemical methods and further confirmation by using Polymerase chain reaction technique (PCR). Carcasses were examined after skinning and evisceration for the total viable count. Swab sampling was used .Viable count was done according to the known conventional methods. The isolation of *Salmonella* was done on enriched and selective media. The samples subjected to the isolation and identification of *Salmonella spp*, about (250) samples included the following :a total of one hundred(100) swabs of slaughtered cattle ,and one hundred samples (100) of ready to eat meat and fifty (50) samples of raw meat in Khartoum state .

At Elkadaro Slaughterhouse the highest contamination level was recorded in evisceration stage at ($4.29+_1.34\log_{10}$ CFU/cm^2), whereas lowest contamination was recorded in the skinning stage ($4.40+_ 0.54$ $\log_{10}$ CFU/cm^2) .At RTM the highest contamination level was recorded ($3.15+_ 2..34\log_{10}$ CPU/cm^2) in shawerma ,whereas lowest contamination was recorded ($2.3957+_0.54\log_{10}$CPU/cm^2) in burger . The highest contamination at butcher shop was ($4.88+_3.54$ $\log_{10}$CPU/cm^2).

From one hundred (100) swabbing samples of slaughtered cattle, 5samples which represented 5% yielded *Salmonella dublin* .

From 100 ready to eat samples (RTM) meat (burger, shawerma) two samples which represented 4% from shawerma yielded *Salmonella dublin*

From fifty 50 fresh raw meat samples ten samples which represented 20% yielded *Salmonella dublin* and *Salmonella typhi*

In conclusion the conventional method and polymerase chain reaction showed that out of 250 samples, 17 samples were found to be contaminated with *Salmonella spp* , 5(10%) swabbing samples slaughtered cattle, 2(4%) samples from ready to eat (RTM) meat and 10(20%) samples fresh raw meat . The results presented in this study indicated the potential risk of contamination of fresh raw meat cattle, ready to eat (RTM) meat cattle products by *Salmonella spp.*

المستخلص

الغرض من هذه الدراسه تقدير التلوث ببكتيريا السالمونيلا من الابقار المذبوحه بالسلخانه ولحوم الابقار الطازجه واللحوم الجاهزه للاكل بولاية الخرطوم ،السودان. بالعد البكتيري و بعزل البكتيريا السالمونيلا من اللحوم الابقار المذبوحه الطازجه ومنتجات الابقار الجاهزه للاكل في ولاية الخرطوم . واخذت العينات من المطاعم والجزارات ومن مسلخ الكدرو وحللت باستخدام الطرق التقليديه والبيوكيميائيه وتأكيد تشخيصها باستخدام التقنيه الجزيئيه

تم جمع مائه عينه مسحه من السلخانه ومائه عينه مختلفه من منتجات اللحوم الجاهزه للاكل (بيرقر والشاورما) وخمسين عينه مختلفه من اللحم الطازج بالجزارات. العينات جمعت من المطاعم المختلفه وسلخانة الكدرو وجزارات بحري بولاية الخرطوم . الذبائح اختبرت بعد السلخ والاحشاء للعد الكلي للبكتيريا .العينات اخذت بطريقة المسحات ،العد الكلي للبكتيريا اجري بالطرق التقليديه.

عزل بكتيريا السالمونيلا تم في الاوساط الداعمه للنمو والانتقائيه.

العينات التي اخذت للعزل 250 عينه كانت كالاتي 100عينه من السلخانه و100 عينه من اللحوم الجاهزه للاكل (البيرقر والشاورما) و50 عينه من الجزارات ، في سلخانة الكدرو اعلى عد بكتيري كان في مرحلة الاحشاء بينما اقل في مرحلة السلخ وكذلك اظهر العد البكتيري اعلى في الشاورما بينما اقل في البيرقر وكان اعلى شي في مرحلة الجزارات.

أظهرت نتائج طريقة العزل التقليديه تلوث 5عينات (5%) بجراثيم السالمونيلا من اصل 100 عينه من السلخانه وكان توزيع عترات السالمونيلا كمايلي جراثيم السالمونيلا دوبلين . تلوث 2عينه من اصل 100عينه من منتجات لحوم الابقار الجاهزه للاكل وكان توزيع عترات السالمونيلا كما يلي سالمونيلا دوبلين. 10عينات من اصل 50 عينه من اللحوم الطازجه وكان توزيع عترات السالمونيلا كمايلي :سالمونيلا دوبلين وسالمونيلا تايفي وخلاصة نتائج العزل التقليديه والتجزيئيه كانت كلاتي:تلوث 17 عينه بجراثيم السالمونيلا من اصل 250 عينه ،تلوث 5 من اصل 100 مسحة عينه من السلخانه بجراثيم السالمونيلا ،وتلوث 2من اصل 100 بجراثيم السالمونيلا من اللحوم الجاهزه للاكل ،وتلوث 10 عينات من اصل 50 عينه من اللحوم الطازجه بجراثيم السالمونيلا .

ان النتائج الممثله في هذه الدراسه تبين الخطوره المحتمله من تلوث اللحوم الطازجه والجاهزه للاكل بجراثيم السالمونيلا

Introduction

The importance of food as a vehicle for the transmission of many diseases has been documented for a long time especially in the developing countries where hygienic standards are not strictly followed and Enforced. The presence of the microorganisms can lead to many food-borne outbreaks. Meat an excellent source for growth of many notorious microorganisms such as mesophilic and psychrophilic bacteria those can cause infection in human, spoilage of meat and economic loss and major source of these deteriorative changes being microorganisms, this renders the meat unacceptable and unfit for human consumption (Kalalou *et al* , 2004 ; Ajiboye *et al* .,2011) . The presence of foodborne pathogens in meat and meat products can result in a range of human health problems as well as economic losses to producers due to recalls from market places (Sofos , 2008). Ready-to-eat (RTE) food products are those foods that do not require further heat treatment to significantly reduce the microbial load before consumption (Conner etal.,2001) and are known to be good growth substrates for pathogenic microorganisms such as *Listeria monocytogenes* (Zhu *et al* .,2005). The presence of a microbiological hazard such as *Salmonell*a, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in RTE meat products is a major concern to food control authorities worldwide. These foodborne pathogens can cause severe illnesses or death to humans, especially high-risk individuals. Major Foodborne pathogens (31pathogens) cause an estimated 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 deaths each year in the United States. Fifty percent of the deaths result from consumption of foods contaminated with *Salmonella*, *L. monocytogenes*, or *E. coli* O157:H7 (Rahn *et al*.,1992). *Salmonella, L. monocytogenes, and E. coli* O157:H7 have been isolated from various types of RTE meat products in the Mediterranean region

(Cabedo *et al*.,2008 ., Osaili *et al*.,2001; Kayisoglu *et al*.,2003, Ulukanli *et al*.,2006). In Turkey, Ulukanli *et al* (2006) isolated *E. coli* O157:H7 from cooked doner (11.3% of samples) and Kayisoglu *et al* (2003) isolated *Salmonella* from cooked beef doner (40% of samples) and cooked chicken doner (80% of samples). In Lebanon, Harakeh *et al* (2005) isolated *Salmonella* (7.4% of samples) and *E. coli* O157:H7 (7.4% of samples) from meat pies and shawirma. In Amman, Jordan, Osaili *et al* (2011) isolated *L.monocytogenes* from shawirma (13.3% of samples) and precooked frozen chicken burgers (76.7% of samples). Contamination of RTE meats by pathogenic bacteria has been previously reported in Trinidad. A voluntary recall by one manufacturer in Trinidad in 2003 due to contamination of *L. monocytogenes*, but other organisms were detected in finished meat products at the plant, including *E. coli, Salmonella spp*., *Campylobacter spp*., and unacceptable levels of aerobic bacteria (Gibbons *et al* 2006). The occurrence of pathogenic microorganisms in RTE meats in Trinidad indicates the need for improved quality assurance by local producers in order to reduce consumers' risks of exposure to infectious foodborne agents. Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*Salmonella enterica*, and *S. bongori*). *Salmonella* has been identified as an important food and water-borne pathogen that can infect human and animals resulting in significant morbidity and mortality (Akkina *et al*., 1999). *Salmonella* is a facultative anaerobe, Gram-negative rod shaped, 2 – 3 x 0.4 – 0.6 μm in size and motile by peritrichous flagella except for *S.Gallinarum* and *S. Pullorum* which are immotile belong to the family enterobacteriaceae (Yang *et a* ., 2003) and it causes food poisoning in the world. . They are urease and Voges-Proskauer negative and citrate utilizing (Montville and Matthews, 2008).

Salmonella are typically non-lactose, non-sucrose fermenting but are able to ferment glucose, maltose and mannitol with the production of acid only as in the case of *S. Typhi* and acid with H2S in the case of *S.Paratyphi* and for most other *Salmonella* serovars (Cruickshank , 1975). Optimum temperature for growth is in the range of 35 – 37ºC but some
can grow at temperatures as high as 54ºC and as low as 2ºC (Gray and Fedorka-Cray, 2002). *Salmonella* grow in a pH range of 4 - 9 with the optimum being 6.5 – 7.5. They require high water activity for growth (> 0.94) but can survive at of < 0.2 such as in dried foods. Inhibition of growth occurs at temperatures < 7ºC, pH < 3.8 or aw < 0.94 (Hanes , 2003). Horgan (1947) made the first report on *Salmonella* infections in cattle. He investigated a food poisoning outbreak at Wad Madani town and isolated *Salmonella* serovar *dublin* from feces of two persons who fell sick after eating meat. Cattle are a major reservoir for *Salmonella* which is carried in the intestinal tract of healthy animals and excreted in feces (Chapman *et al*., 1993). Local Slaughter house environment is observed conducive for the growth of microorganisms, which can rapidly render the meat unsafe for human consumption. The poor hygiene and sanitation prevailing in the abattoirs as well as the shops encourage microbial contaminations and growth. The higher microbial load in the shops further enhances the chances of early meat spoilage (Sudhakar *et al*., 2007). Cross contamination of carcasses with *Salmonella* can also occur during slaughtering operations. Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant [Baird-Parker ,1990, Isaacsonr *et al* 1999].. The behavior of worker was an important thing in the contamination was reported by Elamine (2002) and Jeffery *et al* (2003) and their result indicated that the sources of

meat contamination include the hands and arms of meat handlers, equipment's and contact surface.

Felsenfeld et *al* (1950) were first to examine *Salmonella* incidence in carcass. They sampled from loin area 7% and tenderloin 8% . from Egypt Floyd *et al* (1953) indicated that was only about 8.4 from hog carcasses. Information is available on the incidence of *salmonella* in beef carcasses Other investigation reported incidence in beef ranging from 12% to 35% (Elis,1962 ; cherry *et al*.,1943) . Wilson *et al* (1962) found raw pork products to be highly contaminated.

The contamination of equipment, material, and workers' hands can spread pathogenic bacteria to non-contaminated carcasses.

Many studies on the microbiological hygiene of cattle at slaughter have shown that hide contamination is strongly correlated with carcass contamination, which is likely the result of cross-contamination (inter- and/or intra hide-to carcass contamination) during processing (Arthur *et al*., 2007).

Objectives:

General objective:

- To evaluate the bacteriological status of the cattle carcasses in Elkadrow Slaughterhouse, RTM and raw meat and to isolate and identify *Salmonella spp* using conventional and molecular PCR technique.
- To determine of the bacterial total viable count in beef.

Special objective:

- Detect and characterize *Salmonella spp* in Cattle meat and RTM cattle meat products in Khartoum –Sudan.
- To genotyping of the isolated *Salmonella Spp* using PCR molecular technique.

Chapter one

Literature Review

1.1 Definition:

Food borne salmonellosis is still today a serious public health issue: very common in poor developing countries, due to the bad general hygiene conditions and usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Arroyo and Arroyo.,1995; Alemayehu *etal* .,2002). It is estimated that *Salmonella* is responsible for approximately 93.8 million human cases, with 155,000 deaths annually worldwide (Majowicz *et al*., 2010). The real number of infections is probably significantly higher due to misdiagnosis and under reporting of gastrointestinal illnesses (Voetsch *etal*., 2004).

Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*Salmonella enterica*, and *Salmonella bongori*) . Human salmonellosis is one of the most common and economically important zoonotic disease .The disease can affect all species of domestic animals; young animals and pregnant and lactating animals are the most susceptible. Enteric disease is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicemia, abortion, arthritis and respiratory disease, may be seen. Many animals, especially pigs and poultry, may also be infected but show no clinical illness (Wray *et al*.,2000). Such animals may be important in relation to the spread of infection between flocks and herds and as sources of food contamination and human infection (WHO, 2000).

The course of infection, the clinical signs, the post-mortem findings and epidemiological patterns vary according to the serovar and the animal species involved. Some serovars only affect certain hosts, e.g. *Salmonella gallinarum* in poultry or *S. choleraesuis* in pigs, although most serovars

may cause disease in a wide range of animal species (Snoeyenbos , 1994). Many serovars, including some that are host adapted such as *S.choleraesuis* and *S. dublin*, have been shown to cause disease in humans, and animal attendants, veterinarians and abattoir workers may be infected directly during the course of their work, as may laboratory personnel (WHO , 2000) .Information on the development of appropriate measures for the prevention and control of food-borne diseases, including *Salmonella* infections of humans. The most common vehicles of infection are eggs and egg products, poultry meat and meat from other food animals, and meat products. Contaminated salad crops and spices have also been involved in numerous outbreaks . *Salmonella enteritidis* and *S. typhimurium* are the most wide spread serovars in many European countries, (although *Salmonella* is rare in livestock production, some EU countries have strict control programmes) , while *S. typhimurium* is the dominant serovar in North America (WHO,2000).

1.2 History

Genus *Slamonella* was named in 1900 after U.S. Department of Agriculture (USDA) bacteriologist .The nomenclature of *Salmonella* has undergone many changes within the past decades (Euzéby, 1999; Brenner *et al*., 2000; Tindall *et al*., 2005). Salmon `(1884) who was the first to describe amember of the genus , first isolated the bacterium from porcine intestine as a common cause of hog cholera and designated the type strain *Bacillus choleraesuis* (Smith, 1894), *S.cholerae–suis* (Doyle *et al*., 1997), which he thought caused hog cholera. It was discovered the virus caused hog cholera and Salmon's bacterium by incident of isolate (Doyle *et al* .,1997). In the 1920s and 1930s, Kauffmann (1966) introduced the method for antigenic identification of the *Salmonella* group. According to this Kauffmann-White scheme, each *Salmonella* serotype is recognized by its possession of a particular lipo poly saccharide (LPS) or O antigen and a flagellar or H

antigen. This led to the description of more than 2500 serotypes at present (Brenner *et al.*, 2000; Popoff *et al.*, 2004). The extensive study of the organism has led to the recognition that *Salmonella* is one of the most common causes of human gastroenteritis.

1.3 **A etiology**

Salmonellosis disease is caused by genus of *Salmonella* which belong to enteric bacteria gram negative rod shaped non-spore forming with diameter around .07to 1 .5 µm, lengths from 2 to 5 µm, and flagella that grade in all directions, facultative an aerobe (Doyle *et al*., 1997), *Salmonella* ferment glucose, usually with gas production ,but not ferment lactose or sucrose (Frazier,1958).

1.4 Biochemical Features

Salmonella spp are negative for indole , voges-prousker , phenyl alanine and urease . Most species are motile with peritrichous flagellae except *S.pullorum* gallinarum. *Salmonella* is heat labile so the organism can be inactivated at ordinary cooking temperatures (> 70 °C) although the cooling time and values for temperature and time could change depending on the serotype and the food matrix. In addition *Salmonella* has been shown to tolerate up to 20% salt concentration (Bell and Kyriakides ,2002; Guthrie,1991). Under freezing conditions (from -23°C to -18°C) this microorganism is able to survive as long as seven years (Bell and Kyriakides, 2002). The difficulty in controlling *Salmonella* is due to its ability to survive extreme environmental conditions (Guthrie,1991) The biochemical characteristics of *Salmonella* indicate that they are able to reduce nitrates to nitrites, produce gas from glucose (not always), produce hydrogen sulfide on triple-sugar iron agar, and they are usually able to use citrate as the sole carbon source

1.5 Classification:

1.5.1Taxonomy and characteristics:

According to the latest nomenclature the genus *Salmonella* belongs to the large family of *Enterobacteriaceae*, which reflects recent advances in taxonomy (Popoff ,2001) . The taxonomy and the nomenclature have been the subject of debate in the past decennia. Nowadays, it is generally accepted that the genus *Salmonella* consists of three species, namely *Salmonella enterica*, *Salmonella bongori* and the recently discovered species *Salmonella subterranean* (Shelobolina *et al.*, 2004; Heyndrickx *et al.*, 2005; Tindall *et al.*, 2005). *Salmonella enteric* is subdivided into six sub species, which are distinguishable by certain biochemical characteristics and some of which correspond to the previous subgenera. These subspecies are:

Original subgenera Current nomenclature

- Subspecies I = subspecies *enterica*
- Subspecies II = subspecies *salamae*
- Subspecies IIIa = subspecies *arizonae*
- Subspecies IIIb = subspecies *diarizonae*
- Subspecies IV = subspecies *houtenae*
- Subspecies VI = subspecies *indica*

As mentioned, more than 2500 serotypes are currently described. Historically, serotypes were considered as species and therefore the serotype names were italicized. Nowadays, the former known *Salmonella enteritidis* is written as *Salmonella enteric* subsp. *enteric* serotype *enteritidis* or simply *Salmonella enteritidis*. Serovars belonging to the subspecies *enteric* are mainly associated with mammalians and birds, whereas the other serovars are mainly isolated from non-mammalians, vertebrates or from the environment (Brenner *et al*, 2000).

For the serovars of *S. bongori*, the symbol V was retained to avoid confusion with the serovar name of *S. enteric* subsp. *enterica*. Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipo polysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme; currently approximately 2500 serovars are recognized (Popoff ,2001). This number is constantly being increased. The most common serovars that cause infections in humans and food animals belong to subspecies *enterica*. The serovars of the other subspecies are more likely to be found in poikilo thermic (cold-blooded) animals and in the environment, but are occasionally associated with human disease. Some serovars of subspecies *arizonae* and subspecies *diarizonae* have been associated with disease in turkeys and sheep and others may be carried by free-living or captive reptiles and amphibian .Names are retained only for subspecies *enteric* serovars. These names must no longer be italicized . The first letter is a capital letter. In clinical practice the subspecies name does not need to be indicated as only serovars of subspecies *enteric* bear a name, e.g. *typhimurium. , london or montevideo* are serovars of subspecies *enterica*. The genus *Salmonella* followed by the serotype name may be used for routine practice (e.g. *Salmonella typhimurium*). Most serovars of the other subspecies are designated by an antigenic formula, including subspecies designated by Roman numerical (e.g. *Salmonella* IV 48:g.z51). Up to the present, *Salmonella* bacteria were named according to their pathology, their host and the city where they have been found first and an attention was paid to use an individual name for every bacteria within the same antigen structure in Kauffman-White classification.

1.6 Genome structure

The bacteria can have a combination of three antigens: the O antigen, H antigen, and Vi antigen. The O antigen is located in the cell wall of the bacterium, and each *salmonella bacillus* may posses 2 or more O antigens on it's surface .Also ,the H antigen is a flageller antigen that can be destroyed by heat and enables the motility of the *Salmonella* bacterium (Slack and Snyder ,1978). The last antigen is known as the Vi antigen because this antigen is related the virulence of the bacterium. As a capsular antigen, its presence enhances the virulence of the bacterium that has it—of all the sub-species of *Salmonella*, only two, *Salmonella enteric* serovar *S. typhi* and *S.choleraesuis,* have the Vi antigen (WHO,2005). Depending on whether the type of *Salmonella* has one of two antigens, the bacteria are either monophasic or diphasic, since the bacteria only produce one antigen at a time, each in a certain phase. Those that have only one set of antigens are monophasic—i.e. they have only one phase of antigen production—while those that have two sets of antigens are diphasic—i.e. they have two phases of production, one for each set of antigens (Slack and Snyder,1978).

1.7 Host range of *Salmonella. enterica* serovars

Salmonella serovars can be subdivided into three groups on the basis of host prevalence and pathogenic hazard (Wallis and Barrow, 2005). They can differ substantially in clinical manifestations, ranging from an asymptomatic state to severe illness (Jones *et al*., 2008). Serovars of the first group are known to be highly host-adapted, causing systemic disease in a limited number of related species. The most prominent representative is *S. enterica* serovar *typhi*, which causes typhoid disease only in humans and some non-human primates. *S. enterica* serovar *gallinarum*, is predominantly an avian-adapted serovar, the causative agent of fowl typhoid. The second group consists of host-restricted serovars that cause

systemic disease in specific animals but may also rarely infect other mammals (Kingsley and Bäumler, 2000). For example, *S. enterica* serovar *choleraesuis* causes systemic paratyphoid illness in pigs but infrequently infects humans. Similarly, *S. enterica* serovar *dublin* is usually restricted to cattle, causing systemic disease, but invasive human infections are occasionally reported (Wollin, 2007) and it is capable of causing typhoid fever-like infections in mice (Barrow *et al*., 1994). In contrast, the third group can infect a broad range of avian and mammalian hosts with a wide range of diseases. The most prominent serovars of this group are *S. enterica* serovars *typhimurium* and *enteritidis.* In newly hatched chicks *S. enterica* serovars *enteritidis* and *typhimurium* cause systemic disease and gastroenteritis whereas older chickens are asymptomatic carriers. In calves, *S. enterica* serovar *typhimurium* causes entero colitis including dehydration. In mice, *S. enterica* serovar *typhimurium* causes typhoid fever-like disease (Tsolis *et al*., 1999). Immuno-competent humans often suffer from self-limiting diarrhoea but immuno compromised individuals can develop systemic disease with high mortality rates (Kingsley *et al*., 2009; Dougan *et al*., 2011). Variants of *S. enterica* serovar *typhimurium* are associated with specific avian paratyphoid disease in pigeons and other birds and these may be considered host-adapted (Rabsch *et al*., 2002). A genetic understanding of virulence, host adaptation and host specificity is still poor. Host adaptation can be triggered by the specific organization of the immune system in birds, mammals or cold-blooded vertebrates leading to an adapted pathogenicity gene repertoire of the serovar (Bäumler *et al*., 1998; Kingsley and Bäumler , 2002). The increasing number of available pathogen and host genome sequences combined with specific animal models will doubtless result in new approaches in that field in the future (Suar *et al*., 2006; Dougan *et al*.,2011).

1.8 Epidemiology

According to EFSA epidemiological data (2011), in the European Union (EU) *Salmonella* is the second cause of foodborne disease after *Campylobacter* and it is still first in many EU States, such as Italy. *Salmonella* infections are zoonotic and can be transferred between humans and animals .*Salmonella* bacteria can survive for long periods in the environment. *Salmonella* wide spread in nature occurring in animals ,poultry, insects, swine and environment including water, soil, factory surface ,kitchen surface and raw sea food. Human and animal are directly or indirectly the source of contamination of food with *Salmonella*. The Organism may come from carries , also can come from dogs ,cattle but more important source are poultry and their eggs and rodent. Chickens, turkeys and geese may be infected with a large number of *Salmonella*, which are then found in the fecal matter, in eggs from hens and in flesh of the dressy fowl (Frazier ,1958) and also isolated in and outside of eggs shell. *Salmonella* is spread by the trade of live animals within and between countries .Trade in contaminated animal feed products has also significantly contributed to the spread of *Salmonella* (Sternberg *et al*.,2005; Wierup ,1994) and several large outbreaks in humans have been traced back to contaminated animal feed (Crump *et al*.,2002).However, *Salmonella* is also spread by non-heat-treated meat products. In Sweden, in the 1950s, 500 people were reported to have been infected by *S. montevideo* from meat imported from South America (Silverstolpe *et al*.,1955). Moreover, recent data from Denmark estimate the contribution from imported non-heat-treated meat (duck, turkey, chicken, beef and pork) to human cases of salmonellosis between 13.8% and 26.8%. Many countries have trade restrictions for *Salmonella* and trade between countries has often been interrupted by *Salmonella*-contaminated consignments

(Matthews *.et al*.,2003). There have also been numerous alerts concerning *Salmonella* contaminated meat, meat products and poultry notified through the rapid alert system for food and feed (Rapid Alert System for Food and Feed , RASFF., 2005) . Generally, studies showed that the higher the dose, the higher the probability of becoming ill. Studies on 116 volunteers showed that the lowest dose causing illness was 1 x 105 *S. enterica* serovar *typhi* organisms with 28% attack rate using milk as the vehicle (Hornick *et al*., 1970). However, data from outbreaks often showed that a considerably lower number of ingested organisms caused illness (D'Aoust and Pivnick , 1976). Especially fatty vehicles (chocolate, cheese) may protect salmonellae from the bactericidal action of gastric acidity (D'Aoust, 1994). *Salmonella* is additionally spread between countries by humans as a result of food-borne infections acquired abroad. The overall importance of this route of transmission may reflect the prevalence of *Salmonella* contamination of food (including food of animal origin) in particular country. In low-prevalence countries, such as Finland, Norway and Sweden, > 80% of human cases of salmonellosis are attributed to visits abroad (Anon, 2005). This is in marked contrast to countries such as Denmark and the Netherlands , where roughly the opposite situation exists. In Spain, the most important serotypes causing disease are *Salmonella enteric,* serotypes *enteritidis, typhimurium, haddar, and subsp. I* serotype 4,5,12:i- **(**Usera *et al*.,2001).

1.8.1 Incidence of human salmonellosis and outbreaks

Salmonella enterica subsp. *enterica* accounts for approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Farmer III, 2003). In Germany, since the mid-1990s the reported number of confirmed cases has steadily continued to decrease, with the exception of 2006 and 2007. For 2010, 25,307 human *Salmonella* infections were reported, a

decrease of 19% compared to the 2009 data (RKI , 2011). Twenty-six patients died of salmonellosis. The incidence notification rate in Germany was 30.9 % cases per 100,000 populations and significantly lowers than the average of the last five years (median 63.4% cases per population). Asignificant part of the decrease is explained by declining rates of *S. enterica* serovar *enteritidis* infections since 2001 (Frank *et al*., 2009). However, in 2009 ,58% and 2010, 47% of all infections were still caused by *S. enterica* serovar *enteritidis* . The relative increase is the consequence of decreasing *S. enterica* serovar *enteritidis* cases as well as the rising number of infections caused by the monophasic variant of *S. enterica* serovar *typhimurium* . Since 1999 the National Reference Centre for *Salmonella* and other *enterics* serotyped steadily increasing numbers of isolates from humans belonging to the monophasic variant *S. enterica* serovar *typhimurium* (0.1% in 1999 to 24.9% in 2011). Further serovars causing human salmonellosis are *S. enterica* serovar *infantis* (2%), *derby* (0.8%), *kentucky* and *virchow* (0.5%). All other serovars caused 8.5% of all cases. An outbreak is defined as either a household outbreak, where only members of a single household are affected, or as a general outbreak, where members of more than one household are affected (EFSA and ECDC, 2011). In 2010 for Germany, all together 562 outbreaks with 2,108 cases were reported (RKI, 2011). In three outbreaks more than 40 humans were affected. The largest outbreak involved 110 persons, especially children, and was caused by *S. enterica* serovar *enteritidis* (RKI, 2011). Recently, another large outbreak affected 106 individuals in October/November 2011. The outbreak strain belonged to *S. enterica* serovar *newport*. The vehicle of infection was imported from the Netherlands (Rosner et al., 2012). In Europe, in 2010, 99,020 salmonellosis cases were reported. A 5-year trend (2006-2010) showed a statistically significant decrease in the case numbers (EFSA and ECDC, 2012). The

incidence notification rate was on average 21.5 cases per 100,000 population compared to 23.7 cases per population in 2009 ranging from 1.9 in Portugal to 91.1 confirmed cases per 100,000 population in Slovakia. Sixty-two humans died due to non typhoidal salmonellosis among 46,639 confirmed *Salmonella* cases. The two most commonly reported serovars were *S. enterica* serovar *enteritidis* and *S. enterica* serovar *typhimurium,* representing 45.0% and 22.4% of all reported serovars in confirmed human cases. A decrease was recognized for both serovars in comparison to 2009 (7.3% and 0.9%, respectively). Further *S. enterica* serovars were (1.8%), monophasic typhimurium (1.5%), newport (0.9%), *kentucky* (0.8%), *virchow* and *derby* (each 0.7%), *mbandaka* and *agona* (each 0.5%). Other serovars covered 25.3% of *Salmonella* infections in Europe (EFSA and ECDC, 2012). According to the salmonellosis cases, *Salmonella o*utbreaks within the EU in the years 2007 to 2010 declined sharply from 2,253 to 1,604 outbreaks (verified and possible outbreaks). In 2010, the predominant serovar involved in outbreaks was *S. enterica* serovar *enteritidis* (61.3%). Eggs and egg products were the cause in 43.7% of all strong evidence *Salmonella* outbreaks. Inadequately heat-treated bakery products using raw eggs were the second most frequently known source of *Salmonella* infections (14.4% of verified outbreaks) (EFSA and ECDC, 2012). Similarly, reported data collected between 2001 and 2007 from the World Health Organization Global Foodborne Infections Network including 37 countries showed that *S. enterica* serovars *enteritidis* (43.5%) and *typhimurium* (17.1%) were the most common serovars isolated from humans worldwide (Hendriksen *et al*., 2011). In developing countries, the proportion of S. *enterica serovar enteritidis* decreased from 73.9% in 2001 to 55% in 2007 and in developed countries the proportion of *S. enterica* serovar *typhimurium* decreased from 26.4% to 18.8%. S. enterica serovars *newport* (3.5%), *infantis* (1.8%), *virchow* (1.5%), *hadar* (1.5%) and *agona*

(0.8%) were also frequently isolated (Hendriksen *et al*., 2011). However, regional differences in prevalence of *Salmonella* serovars have been observed. For example, *S. enterica* serovar *heidelberg* was much more frequently reported from North America (top 4) than from Europe (top 9) and Latin America (top 19) and did not occur in the African or Asian region among the 20 most common serovars.

1.8.2 Incidence of Beef salmonellosis and outbreaks

Throughout the last couple of decades several *Salmonella* outbreaks have been associated with contaminated beef (Dechet *et al.*,2006; Stop forth *et al* .,2006; Greig and Ravel, 2009.,). Over the years there has been an increase of *Salmonella* serotype *typhimurium* DT104 infections. All the outbreaks associated with these serotype were associated with dairy products and contact with animals (Dechet *et al*.,2006). An outbreak of multidrug-resistant *Salmonella enterica* serotype *typhimurium* definitive Type DT 104 linked to commercial ground beef in the Northeastern U.S. in 2003–2004 was investigated by Dechet *et al* (2006). A total of 58 patients were identified in 9 states by pulse-field gel electrophoresis (PFGE) (Dechet *et al*.,2006). Isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfa methoxazole, and tetracycline (R-type ACSSuT). Illness was associated with consuming store-bought ground beef prepared as hamburgers at home and with eating raw ground beef . Product trace back linked cases to a single large ground beef manufacturer previously implicated in a multistate outbreak of highly drug resistant *Salmonella enterica newport* infections in 2002 (Dechet *et al*.,2006). Also in 2007, an outbreak of multidrug-resistant *Salmonella serotype Newport* associated to ground beef affected 42 patients in California, *Arizona, Idaho,* and *nevada* (Schneider *et al*.,2011). The patients consumed multiple types of ground beef products purchased at numerous chain store retail locations. These stores had received beef products for grinding from multiple beef

slaughter-processing establishments. Despite detailed ground beef purchase histories both the USDA-FSIS and the California Department of Public Health were unable to identify the source of contamination (Schneider *et al*.,2011). *Salmonella serotype enteritidis* was the most frequent Salmonella *serotype* (991 foodborne outbreaks or 24.1% of the total), followed by *typhimurium* (270 outbreaks or 6.6%) (Greig and Ravel, 2009.). The most frequently reported food categories were 'Multi-ingredient foods', 'Eggs', and 'Produce' and 'Beef' at the third rank (17.0, 14.3, 12.2 and 12.2% of all outbreaks, respectively) (Greig and Ravel, 2009.).

1.7.3 Food borne outbreak

Acute diarrhoeal illness is very common worldwide and estimated to account for 1.8 million childhood deaths annually, predominantly in developing countries (WHO, 2005). Estimates of the burden of food borne diseases are complicated by a number of factors: different definitions of acute diarrhoeal illness are used in various studies, most diarrhoeal illness is not reported to public health authorities, and few illnesses can be definitively linked to food. While not all gastroenteritis is food borne, and not all food borne diseases cause gastroenteritis, food does represent an important vehicle for pathogens of substantial public health significance. Despite the frequent occurrence of illness due to *Salmonella Heidelberg*, only a few published studies have implicated specific food vehicles in human illness (Centers for Disease Control, 1986; Layton *et al*., 1997; Hennessy *et al*.,2004; MacDougal *et al*.,2004). A multisite case-control study of sporadic cases of *Salmonella Heidelberg* illness will guide prevention strategies. Food borne outbreak investigation data reported to the Centers for Disease Control and Prevention from 1973 through 2001 to identify food items that were implicated as the cause of outbreaks of human illness due to *Salmonella heidelberg* and to improve our understanding of sources of *Salmonella heidelberg* infection in the United States

1.9 Virulunce of *Salmonella*:

Understanding the mechanism behind the survival of *Salmonella* bacteria ,as they invade an exposed animal ,and their ability to cause disease would enable researchers to prevent much of suffering and economic losses caused by the pathogen. However, despite substantial research efforts ,progress has been limited. The current Knowledge may be summarized as follows: Following oral uptake, *Salmonella* is successively exposed to low PH in the stomach the strong antimicrobial effects of bile decreasing oxygen supply normal gut flora and metabolites intestinal peristalsis cationic antimicrobial peptides present on the the surface of epithelial cells(Rychlik and Barrow ,2005) .These encounters with stressful environments induce the expression of a number of genes whose products are essential for *Salmonella* to invade the intestinal epithelium and infect the host. The ability to cause disease relies several virulence determinants. Some of these may be seen as virulence determinants in the broad sense, including gene involved in nutrient biosynthesis uptake, stress response (both in and out side the host) and repair of cell damage .These genes may be considered housekeeping genes and are present in other closely related bacteria ,such as *Escherichia coli* (Baumler *et al*.,2000) .Another group of virulence gene specific for the genus *Salmonella* encode adaptation to overcome host defence mechanism and may be called true virulence determinants .The experition of both groups of virulence gene is regulated in response to environmental signals in the host .The regulatory genes mediating this control may be also considered virulence determinants (Baumler *et al*.,2000). The genetic control of *Salmonella* virulence is not fully known However, both plasmid and chromosomal genes are involved .Many of the virulence gene of *S.enteric* are located on pathogenicity island of chromosome, referred to as *Salmonella* pathogenicity island (SPI).These genes are believed to have been acquired by *Salmonella* from

other bacteria species through horizontal gene transfere (VanAsten and VanDijk , 2005).They include function such as host cell invasion and intracellular pathogensis .Thus 12 different SPI have been described . At least six serovars of *Salmonella (abortusovis, choleraesuis, dublin,enteritidis , gllinarum/pullorum and typhimurium*) harbor avirulence plasmid (although not all isolates of these serovars do).These plasmid vary in size among the serovars. All these plasmids contain the *Salmonella* plasmid virulence (SPV) locus .This locus harbors five genes designed spv RABCD (VanAsten and VanDijk .,2005).

Other virulence factors of *Salmonella* include the production of endotoxins and exotoxins,and the presence of fimbriae and flagellae.The role of these factors in the pathogenesis of *Salmonella spp*. is not fully established (VanAsten and VanDijk , 2005).

1.10 Pathogensis of *Salmonella*:

Three common conditions caused by *Salmonella* are gastroenteritis, enteric fever, and bacteraemia (Gray and Fedorka-Cray ,2002). *S. typhimurium*, *S. enteritidis*, and *S. newport* are serotypes associated with human and animal gastroenteritis, *S. typhi* and the paratyphoid species are associated with human enteric fever, and *S. cholerasuis* is associated with bacteraemia in pigs (Gray and Fedorka-Cray ,2002). *S. cholerasuis* is found mostly among animals other than humans, yet it is not as deadly in animal hosts as it is in human hosts (Slack and Snyder, 1978; Gray and Fedorka-Cray ,2002).In order to cause disease, the *Salmonella* bacteria, in general, are first ingested and then travel through the digestive system to reach the small intestine. Within the small intestine, they generate a inflammation of the intestinal cells that leads to the gastroenteritis that is typical of *Salmonella* (Slack and Snyder ,1978). In the case of *S. typhimurium*, the bacteria attack the small intestine by changing the natural architecture of the surfaces of the intestinal cells. Adhesions on the surface of each bacterium bind to receptor

sites on the membrane of the intestinal cells, locally stimulating the formation of membrane 'ruffles'. The 'ruffling' enables *S. typhimurium* to be engulfed through induced pinocytosis, a form of endocytosis, and once a single site of entrance has been established, many bacteria can enter the cell (Gray and Fedorka-Cray,2002).In cattle, *S. typhimurium* causes diarrhea, fever, loss of appetite, and decreased body weight. Adults show symptoms earlier than juveniles, but more calves actually display symptoms and die from *S. typhimurium* . Cattle can be healthy carriers of the bacteria for up to 18 months (Gray and Fedorka-Cray.,2002).

1.11 Distribution of *Salmonella*:

Salmonella infections in animals, *Salmonella*-infected animals may or may not develop disease. Those serovars that were initially

observed to cause disease were found to be adapted to specific animal species, that is:

– *Salmonella abortus* ovis (sheep)

– *Salmonella* choleraesuis (pigs)

-Salmonella *gallinarum* (poultry)

– *Salmonella abortus* equi (horses)

– *Salmonella dublin* (cattle).

These serovars cause disease in the species to which they are adapted and are considered less pathogenic to people. However, when humans become infected, the same serovars often cause severe septicaemia (Scientific Committee on Veterinary Measures relating to Public Health SCVPH , 2003).These host adapted serovars primarily cause abortions or severe gastroenteritis in their animal hosts. A group of more frequently isolated serovars, such as *S.typhimurium, S. enteritidis, S. hadar* and *S. infants* (among others), readily affect both humans and animals. In food animals, these serovars manifest themselves clinically through per-acute septicaemia, acute enteritis or chronic enteritis. In the subclinical form of

the disease, the animal may either have a latent infection or be come a temporary or persistent carrier (Quinn*et al*.,2002).The remaining, less frequently isolated serovars can colonise animals, usually without significant clinical signs, but they are all considered capable of causing gastrointestinal infection of varying severity in humans. In most food animal species, *Salmonellae* usually establish a clinically in apparent infection of variable duration, which is significant as a potential zoonosis. However, under various stress conditions, serovars that are usually non-pathogenic may also cause disease in food animal species. No data are available to give the true prevalence of *Salmonella* in animal production or to provide true comparisons between countries. Existing data indicate that the herd prevalence, depending on animal species and region, may vary between 0% and 90% (in swine, cattle and poultry) (European Food Safety Authority EFSA ,2006). Interestingly, Sweden, Finland and Norway have achieved virtually *Salmonella* free animal production as the result of an intervention strategy, implemented some time ago, which proposed zero tolerance for *Salmonella* (EFSA ,2006).

1.12 History of *Salmonella* research in Sudan:

In Sudan, the prevalence of *Salmonella* serovars is not well documented, as salmonellae are not routinely isolated and identified. Only a few studies have been reported by few workers eg, Horgan (1947) made the first report on *Salmonella* infections in cattle. He investigated a food poisoning outbreak at Wad Madani town and isolated *Salmonella* serovar *dublin* from feces of two persons who fell sick after eating meat. Again the serovar *dublin* was isolated from infected calves and from one of the apparently healthy animals (Soliman and Khan, 1959). Asurvey to acertain the incidence rate of *Salmonella* infection in animals was made in Khartoum (Khan, 1970). During the survey, 230 *Salmonella* cultures were recovered from different sources belonging to 63 serotypes. Subsequent *Salmonella*

surveys which have been conducted at Khartoum and Malakal added15 serovars to the list of Sudan. The serovars recorded were: *S. amager, S. derby, S. kandle,S. reading, S. salford, S.adelaide, S. amersfoort, S. bertin, S. chester, S. mushma*r-*haemek, S. muenche, S. muensters, S. newport, S. pomona* and *S. poona* (Khan, 1970). In his attempt to assess the quality of fresh meats in Sudan, SariyEldin (1971) reported the occurrence of *Salmonella wein, S. dublin, S. havana, S. typhimurium, S. senegal* and *S. braenderup. S. dublin* was also isolated from sheep liver (Salih and Ibrahim, 1972). Fifty-eight *Salmonella* strains were isolated from slaughtered chicken in Khartoum North and Omdurman (Yagoub and Mohamed, 1987). The most common serotypes reported were:*S. mons, S. amek* and *S.uganda*. The incidence of *S. dublin* in the mesenteric lymph nodes and feces of sick calves in Kuku dairy cooperative farm, Omdurman and El Obeid slaughter houses was also reported (Saliem , 1987).Forty-five *Salmonella* isolates (not serotyped) were isolated from carcasses, liver, spleen, intestinal contents of chickens from a poultry farm in El Obeid (unpublished data). The isolation of *Salmonella enteric* subspecies *enteric* serotype san-diego from three goats (3.84%) at Omdurman Central Abattoir was reported (El Tom *et al*., 1999). Recently, *Salmonella umbadah* plus 19 new serovars were reported from different sources at Khartoum (Hag Elsafi *et al*., 2009).Mamoun *et al* (1992) isolated 21 *Salmonella* strains from several poultry farms in three different States in the Sudan. *Salmonella eneritidis* was detected in 1.43% of raw milk samples (Yagoub and Mohammed, 1987; Yagoub *et al*., 2005). Yagoub *et al* (2006) isolated *Salmonella paratyphi* A and *Salmonella paratyphi* B from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdurman cities during the period from February to November, 2001. Yagoub (2009) detected *Salmonella sp*p. in 6.2% of fish samples and Hag Elsafi *et al* (2009) detected *Salmonella spp*.

in 3.4% of fecal samples collected from in and around Khartoum state . Saeed and Hamid (2010) confirmed the role of food handlers in the spread and transmission of food borne communicable diseases which includes salmonellosis as they detected pathogens in 30.1% of the food handlers.

1.13 *Salmonella* in cattle:

Salmonella has been widely reported in cattle (McDonough *et al*., 1999), and infected animals may shed the organism in their feces without showing any clinical signs of disease (Gibson, 1965). Therefore, cattle may carry this organism undetected into an abattoir at the time of slaughter .However, relatively few serotypes are associated with cattle, and of these, *Salmonella enterica* subsp. *enterica* serotype *dublin* (*S. dublin*) and *S. enterica* subsp. *enteric* serotype *typhimurium* (*S. typhimurium*) are the most common in the UK and Ireland (Egan *et al*., 1999; El-Hussein *et al*;2010). The presence of *S. typhimurium* in cattle and the consequent cross contamination of beef carcass tissue is of particular concern as this serotype is one of the most common causes of *Salmonella* infection in developed countries (Gomez *et al*., 1997). Cattle are among the known reservoirs of ***Salmonella***, and ground beef has been implicated as one mode of transmission in food-borne outbreaks (Centers for Disease Control **,** 2006; Centers for Disease Control **.** 2002).*Salmonella* can be found in healthy cattle at slaughter (McEvoy *et al*., 2003) and food-borne outbreaks have been associated with the consumption of beef (Shapiro *et al*., 1999;Centers for Disease Control and Prevention ,2002).The quantitative dynamics of Salmonella carriage by beef cattle at slaughter may influence the chance of carcasses contamination which subsequently may influence the risk of human salmonellosis.

1.14 *Salmonella* in food:

Salmonella spp. has been identified as the most important contaminant of food and the leading bacterial agent responsible for food borne outbreaks in several countries (Majowicz *et al.*, 2010). Contaminated animal feed and wild animals (e.g. birds) have been recognized as important entry sites into the food chain in farm livestock (Skov *et al*., 2008). Another source of contamination is the slaughter process of the animals (Bolton *et al.*, 2003). Pork has been identified as another important source for human salmonellosis (EFSA, 2008b; Pires *et al*., 2012). In the EU and many other countries, eggs and egg products are the foods most frequently implicated in human salmonellosis (Hald *et al*., 2004; EFSA, 2012). *Salmonella* can enter the food chain at any point: crop, farm ,livestock feed, food manufacturing, processing and retailing (Wong *et al*., 2002). A number of workers handle animals during slaughter and processing, and contamination is possible when *Salmonella* or any other pathogen is present on the equipment or the workers' hands or clothing. Contamination most often occurs during specific slaughter stages: bleeding, skinning (or de feathering in poultry), evisceration (removal of chest and abdomen contents, also known as gutting) and pre-processing carcass handling. Cattle may be asymptomatically infected with *Salmonella* and beef can be contaminated during slaughter and processing via gastrointestinal content, and by milk during milking. *Salmonella dublin* which is highly pathogenic to humans, is strongly associated with cattle (host-adapted). This makes cattle an important target for *Salmonella* control efforts. foods that have been implicated in salmonellosis out-breaks included ice cream (Vought and Tatini, 1998), roast beef (Shapiro *et al*.,1999), ground beef (McLaughlin *et al* 2006) , fermented sausage (Sauer , 1997) , peanut butter and spread (Burnett *et al*.,2000) .

1.15 Detection of *Salmonella*:

The detection and identification of *Salmonella spp* is time consuming to the food industry (Worcman-Barninka *et al* ,2001). To detect *Salmonella* more rapidly, an alternative method to the conventional culture method was evaluated using polymerase chain reaction (PCR). PCR has been demonstrated to be a very specific and sensitive method for the detection of *Salmonella* (. Baumler *et al*., 1997). In the last decade, there has been a wide interest in the use of the multiplex PCR (mPCR) technique. mPCR approaches have been largely applied to detect different species of several microbial niches, to differentiate closely related species and to recognize single species (Settanni and Corsetti ,2007)

1.15.1 Culture: The culture techniques and media that may work best in a particular diagnostic situation depend on a variety of factors, including the type of *Salmonella*, source and type of specimens, animal species of origin, experience of the microbiologist, and availability of selective enrichment and selective plating media . In recent years a standard method for detection of *Salmonella* from primary animal production has been developed and evaluated, and an ISO-method is now nearly adopted (Mooijmank,2004). The use of semi-solid media for the detection of *Salmonella* Spp. in poultry feces and other matrices (Working document ISO/TC34 SC9 N681 – annex 1, 17.12.204) .

1.15.2 Pre-enrichment media:

The number of *Salmonella* in feces from asymptomatic animals, environmental samples, animal feed and food is usually low, and it is necessary to use pre-enrichment media, such as buffered peptone water or universal pre-enrichment broth, to assist isolation. This may allow the small numbers of *salmonella*, which may otherwise be killed by the toxic effect of enrichment media, to multiply, or it may help to resuscitate *Salmonella* that have been sub lethally damaged, e.g. by freezing, heating,

exposure to biocides or desiccation. Pre-enrichment may not be the best method for isolating less vigorous *Salmonella* strains, such as the host-adapted strains, from feces because of over growth by competing organisms during non selective pre-enrichment (Molbak *et al*.,2006)

1.15.3Enrichment media

Enrichment media are liquid or semi-solid agar media that contain additives that selectively permit *Salmonella* to grow while inhibiting the growth of other bacteria. Some, however, are also relatively toxic to certain serovars of *Salmonella*, e.g. selenite inhibits *S. choleraesuis*, and brilliant green is toxic to many strains of *S. dublin*. Elevated temperatures have also been used to increase the selectivity of enrichment medium, and a temperature of 43°C is used in some laboratories, although this may be inhibitory with some media, e.g. tetrathionate and Rappaport–Vassiliadis at 43°C inhibit temperature-sensitive strains, especially *S. dublin* and 41.5°C is now recommended for incubation of Rappaport–Vassiliadis broth (International Organization for standardization ISO ,2002). Selective motility enrichment may also be used to increase the sensitivity of *Salmonella* isolation and semi-solid enrichment media, e.g. MSRV or diagnostic semi-solid *Salmonella* medium (DIASALM), may provide greater sensitivity (. Voogtn *et al*.,2001). Examples of selective enrichment media are sodium tetrathionate, as in Muller–Kauffman broth, selenite F, selenite cysteine, brilliant green broth and Rappaport–Vassiliadis broths, or semi-solid Rappaport–Vassiliadis medium. In some cases it may be advantageous to use more than one selective broth or to culture by both pre-enrichment and direct selective enrichment/direct plating, although often the benefit does not justify the extra cost. Additions such as Ferrioxamine E may be added to selective media to enhance isolation of *Salmonella* from iron or nutrient-limited samples such as eggs, water or

soil (Reissbrod ,1995) or antibiotics may be added to enhance the isolation of antimicrobial resistant strains .

1.15.4 Selective plating media

These are solid, selective agars that permit differential growth to varying degrees. 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 (H2S) production. The results are read after 24 and 48 hours of culture at 37°C. *Salmonella* form characteristic colonies on such media that are usually distinguishable from the colonies of other bacteria on the plate, with the possible exceptions of *Proteus*, *Pseudomonas* and *Citrobacter*. Lactose-fermenting *salmonella* may occasionally be isolated and H2S production may be variable. Such atypical strains may be more effectively detected when semi-solid selective media are used. Diasalm medium is particularly useful in this respect as presumptive confirmation by slide agglutination testing using polyvalent O, H or specific antisera can be carried out on liquid from the growth zone in the plate. *Salmonella abortus* ovis is a slow-growing serovar and it is usual to incubate plates for up to 72 hours and to use the nonselective blood agar. Examples of selective media are brilliant green agar, xylose lysine desoxycholate agar, deoxycholate/citrate agar, Rambach agar, and bismuth sulphite agar.(Bell and Kyriakides,2002).

1.16 Immunological and nucleic acid recognition methods

These are include electrical conductance/impedence, immunomagnetic separation (IMS), enzyme linked immunosorbent assay (ELISA), gene probes PCR methods, including nucleic acid sequence based amplification (NASBA) (Cook , 2003) and real time (Perelle *et al*.,2004) or quantitative PCR (. Piknoval *et al*.,2005).

1.16.1 Poly Merase Chain Reaction:

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Polymerase Chain Reaction was developed in 1984 by the American biochemist, Kary Mullis. Mullis received the Nobel Prize and the Japan Prize for developing PCR in 1993 (Bartlett ,2003). However the basic principle of replicating a piece of DNA using two primers had already been described by Gobind Khorana in 1971. Progress was limited by primer synthesis and polymerase purification issues (Kleppe *et al*.,1971). PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications (Saiki *et al*.,1985). The polymerase chain reaction is a powerful technique that has rapidly become one of the most widely used techniques in molecular biology because it is quick, inexpensive and simple. The technique amplifies specific DNA fragments from minute quantities of source DNA material, even when that source DNA is of relatively poor quality (Erlich ,1989).PCR; the quick, easy method for generating unlimited copies of any fragment of DNA, is one of those scientific developments that actually deserve timeworn superlatives like "revolutionary" and "breakthrough. From the daily practicalities of medical diagnosis to the theoretical framework of systematics, from courts of law to field studies of animal behavior, PCR takes analysis of tiny amounts of genetic material-even damaged genetic material to a new level of precision and reliability. Furthermore, many important contributions to the development and application of PCR technology have been made; however the present paper is an attempt to review basics of PCR.

1.16.2 Serological tests:

In recent years ELISA (Vanziderveld *et al*.,1992 ; Barrow , 1994) has been developed for the diagnosis of *S. enteritidis* and *S. typhimurium* infections in poultry and for other serovars in farm animals. The ELISA has been used effectively to identify serologically *S. dublin* carrier cattle and can be applied to bulk milk for screening dairy herds.

1.17 Symptoms of *salmonella*:

1.17.1 Symptoms of *Salmonlla* in humans

Symptoms of salmonellosis include diarrhea, fever, and abdominal cramps. They develop 12 to 72 hours after infection, and the illness usually lasts 4 to 7 days. Most people recover without treatment. But diarrhea and dehydration may be so severe that it is necessary to go to the hospital. Older adults, infants, and those who have impaired immune systems are at highest risk.(Monteville and Matthews ,2008)

1.17.2 Symptoms of *Salmonella* in animal

Many animals with Salmonella have no signs of illness at all and appear healthy. Pets that become sick from *Salmonella* infection typically have diarrhea that may contain blood or mucus .Sick animals may seem more tired than usual and may vomit or have a fever. If your pet has these signs of illness or you are concerned that your pet may have *Salmonella* infection. (National Centers for Emerging and Zoonotic Infectious Disease NCEZID, 2017)

1.18 Treatment of *Salmonella*:

In the last 20 years,the world wide emergence of multidrug-resistant *Salmonella* serotypes has become of a great concern. Since the beginning of the1990s, strains of *Salmonella* which are resistant to a range of antimicrobials including first-choice agents for the treatment of humans,

have emerged and are threatening to become a serious public health problem(Threlfall *et al* ., 2000). This resistance results from the use of antimicrobials in both humans, and animal husbandry. Multi-drug resistance to critically important antimicrobials is compounding the problems. Emerging resistance in these pathogens is mainly because of increasing usage of antimicrobial agents in clinics and slaughterhouses and this is becoming a global problem (Adesiyon *et al*., 1989,].The increase isolation of single or multiple resistant *Salmonella* from human infections is due to abundant use of antimicrobial agents in food production(Zhao *et al*., 2006). Remarkable numbers of antimicrobial agents, which are used in treatment of salmonellosis and other bacterial infections in human, are also used in slaughterhouses (Fey *et al* .,2003). *Salmonella* gastroenteritis is usually a self-limiting disease. Fluid and electrolyte replacement may be indicated in severe cases. Because antibiotics do not appear to shorten the duration of symptoms and may actually prolong the duration of convalescent carriage, they are not routinely used to treat uncomplicated non typhoidal Salmonella gastroenteritis. Current recommendations are that antibiotics be reserved for patients with severe disease or patients who are at a high risk for invasive disease .Historically, recommended regimens for the treatment of typhoid fever included ampicillin, trimethoprim-sulfa methoxazole, or chloramphenicol .Presently, quinolone, macrolide, and third-generation cephalosporin antibiotics are preferred for empiric therapy pending sensitivities. Unfortunately, sensitivity to quinolones has been steadily declining, and these are no longer fool-proof agents for typhoid fever. A growing rate of resistance of non typhoidal *salmonella* to nalidixic acid and ceftriaxone has been reported.(Aarestrup ,1999). A study of more than 1000 stored *Salmonella* isolates from Finland has confirmed earlier data that showed that resistance to nalidixic acid by means of disk diffusion

is a sensitive and specific method of screening *Salmonella* isolates for reduced susceptibility to fluoroquinolones.

(Cardoso *et al.*, 2006).Azithromycin is likely to be the preferred empirical treatment, often given together with ceftriaxone, in developed countries where chloramphenicol is usually reserved for life-threatening situations, for which no alternatives are available, and physicians are reluctant to use fluoroquinolones in children and lack easy access to gatifloxacin. (Cardoso *et al*., 2006) .In an endemic area such as Nepal, gatifloxacin is as effective as chloramphenicol in ambulatory young patients, and adherence to treatment is improved by the shorter duration and smaller number of tablets in the gatifloxacin regimen.(Aarestrup ,1999) .*Salmonella* bacteremia is generally treated with a single bactericidal drug for 10-14 days. Given the resistance trends, life-threatening infections should be treated with both a third-generation cephalosporin and a fluoroquinolone until the susceptibilities of antimicrobial agents are known. (Cohen *et al*., 1984).

1.19 Prevention of *Salmonella*:

1.19.1 Prevention of *Salmonella* in humans

There is no vaccine to prevent salmonellosis. Because foods of animal origin may be contaminated with *Salmonella*, people should not eat raw or undercooked eggs, poultry, or meat. Raw eggs may be unrecognized in some foods, such as homemade Hollandaise sauce, Caesar and other homemade salad dressings, tiramisu, homemade ice cream, homemade mayonnaise, cookie dough, and frostings. Poultry and meat, including hamburgers, should be well-cooked, not pink in the middle. Persons also should not consume raw or unpasteurized milk or other dairy products. Uncooked meats should be kept separate from produce, cooked foods, and ready-to-eat foods. Hands, cutting boards, counters, knives, and other utensils should be washed thoroughly after touching uncooked foods (CDC, 2010). Hand should be washed before handling food, and between

handling different food items. People who have salmonellosis should not prepare food or pour water for others until their diarrhea has resolved. Many health departments require that restaurant workers with *Salmonella* infection have a stool test showing that they are no longer carrying the *Salmonella* bacterium before they return to work (CDC,2010) .People should wash their hands after contact with animal feces. Because reptiles are particularly likely to have *Salmonella*, and it can contaminate their skin, everyone should immediately wash their hands after handling reptiles. Reptiles (including turtles) are not appropriate pets for small children and should not be in the same house as an infant. *Salmonella* carried in the intestines of chicks and ducklings contaminates their environment and the entire surface of the animal. Children can be exposed to the bacteria by simply holding, cuddling, or kissing the birds. Children should not handle baby chicks or other young birds. Everyone should immediately wash their hands after touching birds, including baby chicks and ducklings, or their environment. Some prevention steps occur every day without you thinking about it. Pasteurization of milk and treatment of municipal water supplies are highly effective prevention measures that have been in place for decades. In the 1970s, small pet turtles were a common source of salmonellosis in the United States, so in 1975, the sale of small turtles was banned in this country. However, in 2008, they were still being sold, and cases of Salmonella associated with pet turtles have been reported.(CDC,2010). Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated foods. Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination and other food handling errors that can lead to outbreaks. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important

prevention measure. In the future, irradiation or other treatments may greatly reduce contamination of raw meat.(CDC.,2010).

1.19.2 Prevention of *Salmonella* in animal:

Many efforts have been made to find effective vaccines against *Salmonella* infections, especially in cattle and poultry but also in swine .Alive attenuated vaccine against *S.gallinarum* in poultry is available and there is currently demand for a vaccine control Salmonella infections associated with human food poisoning ,in particular, *S. enteritidis* (Feberwee *et al*.,2001). However ,due to the complicated pathogenesis of *Salmonella* infection, no significant breakthrough has been achieved.(Chiu and Chu ,2004).Vaccines to control *Salmonella* infection, especially inactivated vaccines, are in use all over the world .in recent years increasing numbers of live vaccines have been developed but most of them are not yet authorized .Vaccination can play an important role in intervening against *Salmonella* in high-prevalence herds (Haesebrouk *et al*.,2004 ;Lumesden and Wilkie ,1992 ;Springer *et al*.,2001).However ,immunization should not be used but isolation can be conducted with other measure ,such as veterinary hygiene and improved management.

1.20 Hazard analysis and critical control points (HACCP):

HACCP is a systemic preventive approach to food safety and pharmaceutical safety that identifies physical, allergenic,chemical and biological hazards in production process that can cause the finished product to be unsafe, and designs measurements to reduce these risks to a safe level .In this manner, HACCP is referred as the prevention of hazards rather than finished product in spection.The HACCP system can be used at all stages of a food chain ,from food production and preparation processes including packaging ,distribution, etc.

HACCP itself was conceived in the 1960s when the US National Aeronautics and Space Administration (NASA) asked Pillsbury to design and manufacture the first foods for space flights. Since then ,HACCP has been recognized internationally as a logical tool for adapting traditional inspection method to a modern, science-based ,food safety system (International HACCP Alliance 2007).Hence ,HACCP has been increasingly applied to industries other than food, such as cosmetics and pharmaceuticals end. HACCP is focused only on the health safety issues of product and not the quality of the product. Potential biological hazards in meat and poultry include bacteria ,toxin ,viruses ,protozoa and parasites. Of the microbiological hazards ,the most important are bacteria .Bacteria cause a large proportion (approximately 90%) of all food borne illness .Bacteria that cause human illness ,including disease ,are termed pathogenic. The pathogens that are most likely to be found in commonly slaughtered (cattle ,sheep, swine) and poultry (chicken and turkey) include *Salmonella* ,Campylobacter and *Listeria monocytogenes*. Although *Escerichia coli* also is found in livestock and poultry, most forms of *Escherichia coli* are not pathogenic .*Escherichia coli* O157:H7 is pathogenic. The ultimate source for all of these pathogens is apparently healthy animals that shed these bacteria in their feces .While dressing the carcasses during the slaughter process, these bacteria may be transferred from the hide and offal to the carcass causing contamination .All of these pathogens have been implicated in widely publicizes food borne disease outbreaks associated with the consumption of meat and poultry products. proper cooking, fermentation, cooling and storage of food can destroy and or prevent growth of these bacteria (FSIS1999).

1.21 Principles of the HAACP system:

The HACCP system consists of the following seven principles:

1. Conduct a hazard analysis
2. Determine the Critical Control Points (CCPs)
3. Establish critical limit(s)
4. Establish asystem to monitor of the CCP
5. Establish the corrective action to the taken when monitoring indicates that a particular CCP is not under control
6. Establish procedures for verification to confirm that the HACCP system is working effectively.
7. Establish documentation concerning all Procedures and records appropriate to these principles and their application. (FAO Corporate Document Repository, 1997).

Chapter Two

Materials and Methods

2.1. Study area

The study was conducted at Khartoum North, Sudan during October 2016 - March 2017. Samples were collected randomly from Elkadrow Slaughterhouse (n= 100), Restaurants (n=100) and butcher shops (n=50).

2.2. Samples collection

2.2.1. Slaughterhouse

Swabs (n=100) were collected randomly from cattle carcasses after skinning (n=50) from foreleg (n=25), thigh region (n=25) and after evisceration (n=50) from foreleg (n=25), thigh region (n=25).

Swabs moistened with normal saline were rubbed on the carcasses for 30 seconds and transported to the laboratory in an ice box and cultured on the day of slaughtering.

2.2.2. Restaurants

Twenty-five grams of burger (n=50), and shawarma (n=50) were randomly collected from cafeterias and transported to the laboratory under aseptic conditions at 4°C and cultured on the same day of collection.

2.2.3. Butcher shops

Twenty-five grams of meat (n=50) were collected from butcher shops and transported to the laboratory under aseptic and refrigerated (4^0C) and cultured on the day of collection.

2.3 Application of viable count

Test tubes containing swabs were vortexed for 30 seconds for uniform distribution of micro-organism. Also test tubes containing meat samples from both cafeterias and butcher shops were vortexed for 30 seconds for uniform distribution of micro-organism. Ten fold serial dilution up to 10^5

of all samples were prepared using sterile normal saline solution (NSS) and the samples were processed for total viable count (CFU/cm^2) which estimated by standard pour plate method as described by Barrow and Felthem (2003). Dilutions of 10^4 and 10^5 were used .Dilution of each sample were inoculated in dublicate in to the nutrient agar medium . After solidification of agar, the plates were incubated at 37^0C for 24hrs.

2.4. Identification of isolates

Pure isolates were identified biochemically according to Barrow and Felthem (2003).

2.4.1Urease test:

Aslope of urea agar medium was inoculated with tested organism and then incubated and examined after 24 hrs and daily for 7days for the change in color of the medium to pink indicating positive result , while yellow color indicated negative result (Barrow and Felthem , 2003).

2.4.2 Citrate utilization :

The tested organism was inoculated in simmon's citrate medium and then incubated at 30^0C and examined for 7days . Blue coloration was considered positive (Barrow and Felthem , 2003).

2.4.3 Indole test:

Suspected colony was inoculated in peptone water and incubated for 24 hrs at 35^0C .Test for indole was done by adding 0.2-0.3 ml Kovak's reagent ,appearance of distinct red color means positive result and yellow means negative result (Barrow and Felthem , 2003).

2.4.4 Hydrogen sulphide production (kiligler test):

The test organism was inoculated on a tube of triple sugar iron agar by stabbing and streaking the slope; observed daily for up to 7days for blacking of butt only due to H_2S production (Barrow and Felthem , 2003).

2.4.5 Methyl red (MR) and voges-proskauer (VP) reaction:

The tested organism was inoculated on glucose phosphate (MR) medium and incubated at 37^0C for 2 days, 2 drops of methyl red solution were added, shaked and examined. Apositive MR showed colour at the surface, an orange or yellow was regarded as negative .For VP reaction 0.6ml 5% alfa naphthole solution and 0.2 ml 40% KOH aqueous solution were added and the tubes were shaked well then sloped to increase the surface and examined after 15min and one hour. Apositive reaction is indicated by strong red colour (Barrow and Felthem ,2003).

2.4.6 Motility test:

The organism was stab inoculated in tubes of motility medium to a depth of about 5mm and incubated at 37^0C for 24 hrs .Motile organism migrated throughout the medium which became turbid ,while the growth of non-motile organism is confined to the stab inoculums (Barrow and Felthem ,2003).

2.5. Isolation of *salmonella*

2.5.1. Slaughterhouse

Swabs were transferred to selenite f broth , incubated at 37°Cfor 24 h, then a loopful swabs were streaked onto Deoxychoclate agar and incubated aerobically at 37ºC for 24 hrs, plates were examined visually for growth. Pure cultures were cultured into nutrient agar for 24 hrs at 37^0C and stored at 4ºC.

2.5.2. Restaurants and butcher shops

Samples were prepared according to the International Organization for Standardization (IOFS) as described by Mollab and Mosfien (2003). Briefly, 25 gram of meat was homogenized in 225 ml of buffered peptone water (BPW) (Oxoid, England), the suspension was incubated at 37°C for 20 hrs, homogenized samples (1ml) were added to 9ml of selenite F broth for 24 hrs at 37°C, then a loopful of the suspension were streaked on Deoxychoclate agar and incubated for 24 hrs at 37°C, plates were

examined visually for growth. Pure cultures were cultured into nutrient agar for 24 hrs at 37^0C and stored at 4ºC.

and test tubes were sterilized in hot air oven at 160 for one hour.

2.6 Isolation of the bacteria associated with *Salmonella*

2.6.1 Isolation of *E.coli*

2.6.1.1 Slaughterhouse

Swabs (n=100) were collected randomly from cattle carcasses after skinning (n=50) and after evisceration (n=50) .

Swabs moistened with normal saline were rubbed on the carcasses for 30 seconds and transported to the laboratory in an ice box and cultured on the day of slaughtering.

2.6.1.2. Restaurants

Twenty-five grams of burger (n=50), and shawarma (n=50) were randomly collected from cafeterias and transported to the laboratory under aseptic conditions at 4°C and cultured on the same day of collection.

2.6.1.3. Butcher shops

Twenty-five grams of meat (n=50) were collected from butcher shops transported to the laboratory under aseptic and refrigerated (4^0C) and cultured on the day of collection.

2.6.1.4 Processing of Samples

The samples were inoculated on Deoxychocolate agar and incubated aerobically at 37^0C for 24 hrs. The plates were observed for the growth of *E.coli*. Asingle pink isolated colony was picked for the prepration of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. Biochemical tests were performed to confirm the *E.coli* using indole test, urease production, methyle red presumptive test ,vogesproskuar , sugar fermentation on triple sugar iron agar.

2.6.2 Isolation of *Pseudomonas*

2.6.2.1 Slaughterhouse

Swabs (n=100) were collected randomly from cattle carcasses after skinning (n=50) and after evisceration (n=50) .

Swabs moistened with normal saline were rubbed on the carcasses for 30 seconds and transported to the laboratory in an ice box and cultured on the day of slaughtering.

2.6.2.2. Restaurants

Twenty-five grams of burger (n=50), and shawarma (n=50) were randomly collected from cafeterias and transported to the laboratory under aseptic conditions at 4°C and cultured on the same day of collection.

2.6.2.3 Butcher shops

Twenty-five grams of meat (n=50) were collected from butcher shops transported to the laboratory under aseptic and refrigerated (4^0C) and cultured on the day of collection.

2.6.2.4Processing of Samples

The samples were inoculated on Deoxychocolate agar and incubated aerobically at 37^0C for 24 hrs. The plates were observed for the growth of *Pseudomonas*. Asingle isolated colony was picked for the prepration of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. Biochemical tests were performed to confirm the *Pseudomonas* using indole test, urease production, methyle red presumptive test, vogesproskuar , sugar fermentation on triple sugar iron agar

2.6.3 Isolation of *Proteus*

2.6.3.1 Slaughterhouse

Swabs (n=100) were collected randomly from cattle carcasses after skinning (n=50) and after evisceration (n=50).

Swabs moistened with normal saline were rubbed on the carcasses for 30 seconds and transported to the laboratory in an ice box and cultured on the day of slaughtering.

2.6.3.2. Restaurants

Twenty-five grams of burger (n=50), and shawarma (n=50) were randomly collected from cafeterias and transported to the laboratory under aseptic conditions at 4°C and cultured on the same day of collection.

2.6.3.3. Butcher shops

Twenty-five grams of meat (n=50) were collected from butcher shops transported to the laboratory under aseptic and refrigerated (4^0C) and cultured on the day of collection.

2.10.3.4 Processing of Samples

The samples were inoculated on Deoxychocolate agar and incubated aerobically at 37^0C for 24 hrs. The plates were observed for the growth of *Proteus*. Asingle isolated colony was picked for the prepration of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. Biochemical tests were performed to confirm the *Proteus* using indole test , urease production, methyle red presumptive test ,vogesproskuar , sugar fermentation on triple sugar iron agar

2.7 Polymerase Chain Reaction (PCR)

2.7.1. DNA extraction

DNA extraction was done according to Salehi *et al* (2012). Briefly, a single colony of the isolates (n=5) were suspended in 50µl distilled water (DW). The suspension was vortexed, boiled for 20min, transferred to Eppendorf tube and incubated at 20°C for 2min, centrifuged (11600xg) for 10min, the supernatant was preserved at 4°C until it was used. After vortexing, the suspension was boiled for 20min in water bath, transferred tubes were incubated at -20°C for 20min and taken to water bath for 2min,

centrifugated to (11600xg) for 10min. The aqueous phase was transferred to Eppendorf tube, preserved at 4°C until used.

2.7.2. Duplex PCR

Amplification was performed with 5µl of DNA sample, 25µl of Go Taq Green Master Mix (Intron biotechnology koria), 2µM of each primer (Table 1), 15µl of DNase/ RNase free water in a final volume of 50µl. The reactions were performed in a DNA thermo cycler (Techne, cyclogene, UK). The m-PCR protocol consisted of the following steps was done: initial denaturation of 5min at 95°C; 40 cycles, with considering of 1 min at 95°C, 1min at 60°C and 30 seconds at 72°C; and a final extension step of 10min at 72°C (Stegniy *et al* .,2014).

Table 1: Oligonucleotide primer sequences used for detection of *Salmonella spp*

Species	Primer	5*-3*	Amplicon size (bp)
Salmonella spp.	Salm 3 Salm4	GCTGCGCGCGAACGGCGAAG TCCCGCCAGAGTTCCCATT	387
Salmonella enterica ser. Enteritidis	Sent F Sent R	AAATGTGTTTTATCTGATGCAAG AGG' GTTCGTTCTTCTGGTACTTACGA TGAC	299
Salmonella enterica ser. Typhimurium	Styp F Styp R	CCCCGCTTACAGGTCGACTAC AGCGGGTTTTCGGTGGTTGT	433
Salmonella enterica ser. Typhi	Styphi_F Styphi_R	CACGCACCATCATTTCACCG AACAGGCTGTAGCGATTTAGG	738
Salmonella enterica ser. Dublin	Sdub_R Sdub_F	ACGCGAAATCTGATGGTCTT GCCCACCAGTTGTGAAAGGC	203
Salmonella enterica ser. Gallinarum-Pullorum	Sgal_F Sgal_R	CCGCACAACACATCAGAAAG AGCTGCCAGAGGTTACGCTG	97

2.7.3. Gel electrophoresis

The agarose gel electrophoresis detection was followed as described by Sambrook *et al* (1989) .Five µL aliquots of the sample after PCR was analyzed by using 1% (w/v) agarose gel electrophoresis using 1X TBE running buffer , stained with 3.0 µL ethidium bromide(10mg/ml) (promefa USA) (Zhou and jiao ,2005), (Jalali and Abedi ,2008) and photographed under UV trans-illuminator then documented with a gel documentation apparatus (Gel Doc 2000,BIO-RAD,USA).

2.8 Statistical analysis

The data obtained were analyzed by ANOVA using statistical package for the social sciences (SPSS). Statistical significance was set at a P value of <0.05.

Chapter Three

Results

3.1. Bacterial total viable count

3. 1.1. Total viable count of bacteria in Beef meat at Elkadrow slaughterhouse

The mean total viable count in skinning stage ranged from 4.40+_ 0.54 log_{10} CFU/cm^2 and 4.29+_1.34log_{10} CFU/cm^2 in the stage of evisceration. At the skinning stage, bacterial counts range was from 1.22+_0.8log_{10} CFU/cm^2 on the foreleg and 1.85+_1.8log_{10}CFU/cm^2 in the thigh region. At evisceration viable count range was 2.22+_1.80 log_{10}CFU/cm^2 on the foreleg and 2.85+_1.58log_{10}CFU/cm^2 on the thigh region (Table 1).

3.1.2. Total viable count of bacteria at Ready to eat Meat

The mean total viable counts were 2.40 +_0.54log_{10}CPU/cm^2 in burger and 3.15+_2.34log_{10} CPU/cm^2 in shawerma (Table2). The mean total viable count in shawerma was higher than burger.

3.1.3. Butcher shops

The mean total viable count for raw meat was 4.88+_3.54 log_{10}CPU/cm^2 (Table 2).

3.2. Isolation and identification of *Salmonella*

3.2.1. Slaughterhouse

Five isolates of *Salmonella spp.* were isolated from 100 swabs (50swabs after skinning and 50 swabs after evisceration) which represented 5%. Those swabs were collected from cattle carcasses at Elkadrow slaughterhouse (Table3). The result showed black centered colonies surrounded by a clear zone which indicated *Salmonella's* colonies. Figure (1) showed the percentage of *Salmonella spp.* isolates. After skinning, one isolate was found out of the 50 swabs which represented 2%. And four isolates were found out of the 50 swabs after evisceration which

represented 8%. The result showed that *Salmonella spp*. percentage was higher after evisceration samples.

3.2.2. Restaurants

Two isolates of *Salmonella spp.* were isolated from 100 samples (50samples from shawerma and 50 samples from burger) which represent 2% (Table 4). Figure (2) showed the percentage of *Salmonella spp.* isolates. Two isolates of *Salmonella spp.* were found out of the 50 samples from shawerma which represented 4 %. No isolate of *Salmonella spp.* was found out of the 50 samples from burger.

3.2.3. Butcher shops

Ten isolates of *Salmonella spp* .were isolated from 50 samples of fresh raw meat. These ten isolates represent 20% of the samples (Table 5). Figure 3 showed the percentage of *Salmonella spp.* isolates. The result shows that the percentage of *Salmonella spp.* was higher in Butcher shops' s raw meat samples than Restaurant's product

Table 2. The mean total viable count of bacteria ($log_{10}CFUcm^2$) at different operation points at different sites on carcasses in Elkadarow slaughterhouse

site	No of Samples	processing		Significance
		Skinning	Evisceration	
Foreleg	50	1.22+_0.8log_{10} CFU/cm^2	2.22+_1.8log_{10}CFU/cm^2	*
Thigh region	50	1.85+_1.8log_{10}CFU/cm^2	2.85+_1.58log_{10}CFU/cm^2	*
Total	100	4.3954+_0.54	4.29+_1.34log_{10}	*

Table 3. The mean total viable count of bacteria ($log_{10}CFUcm^2$) in Burger and Shawerma and Butcher shop (raw meat).

Type of processed meat	No of Samples	Mean +_ Standard Definition ($log_{10}CPU/cm^2$)	Significance
Burger	50	2.3957+_0.54	*
Shawerma.	50	3.15+_2..34	*
Butcher shop	50	4.88+_3.54	*

Table 4. The number of *Salmonella spp.* isolated from Elkadarow Slaughterhouse

Location		Number of samples	No of positive samples	percentage
Slaughterhouse		100samples	5	5%
	After skinning	50	1	2%
	After evisceration	50	4	8%

Table (5) The number of *Salmonella spp.* isolated from Restaurants product (shawerma and burger)

location		Number of samples	No of positive samples	percentage
Restaurants	Shawerma	50	2	4%
	Burger	50	0	0

Table (6) The number of *Salmonella spp.* isolated from butcher shop's raw meat

Location	Number of samples	No of positive samples	percentage
Butcher shops	50 samples	10	20%

3.3 Identification of *Salmonella spp*

As shown in (table 6) the isolated *Salmonella Spp* bacteria behaved similar to the scheme performed by Barrow and Felthem (2003) for the isolation and identification of microorganism .This indicated that the organism isolated were related to enterobacteriace. Further identification was undertaken by various biochemical tests (table 6) .Identification revealed that all the isolates were Salmonella spp.

Table 7. The Primary tests and Biochemical tests used for identification of *Salmonella spp*

Test	**Result**
Citrate	+
shape (gram stain)	+
Deoxy chocolate agar (DCA)	+
Urease	-
Kligler	+
VP	-
Methyl red	+
Motility	+

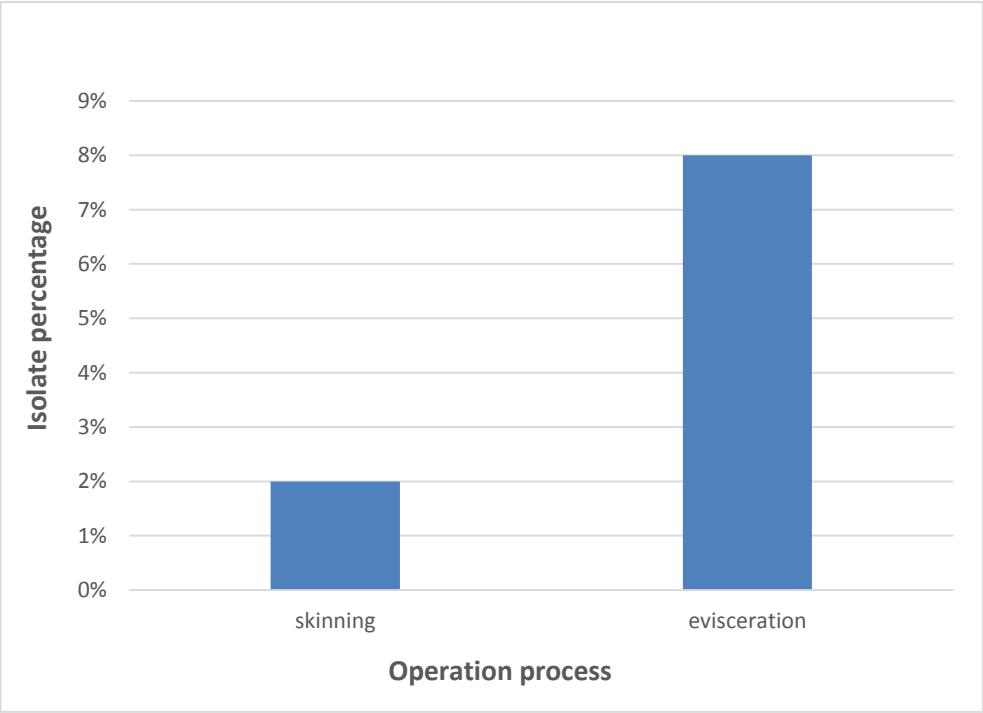

Figure 1. Percentage of *Salmonella spp*. after skinning and after evisceration

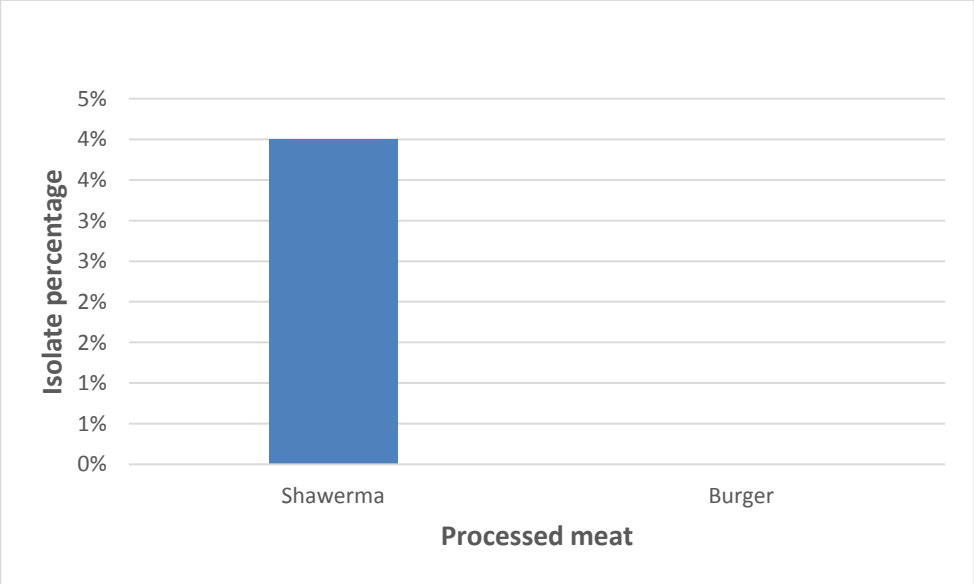

Figure 2. Percentage of isolated *Salmonella* in restaurants products

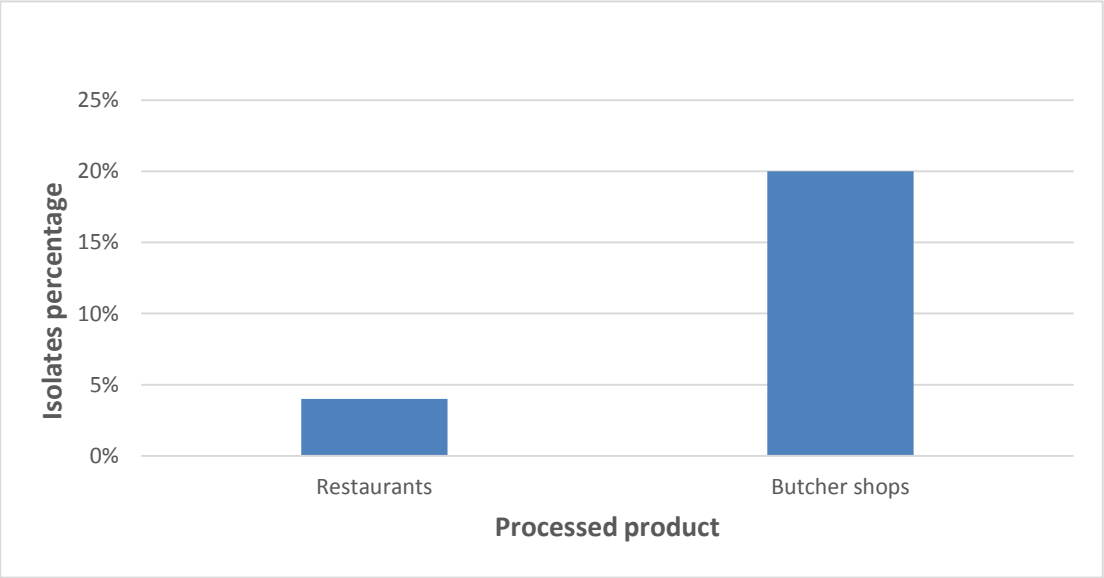

Figure 3. Percentage of *Salmonella* isolates in butcher shops and restaurants

3.4. Isolation of Associated aerobic bacteria:

In association with *Salmonella spp.* multiple other aerobic bacteria were isolated. these organisms are; *E.coli, Pseudomonas spp. and Proteus spp.* (Table 7) .

Figure 4 shows the result after skinning in the slaughterhouse isolates. *E.coli* was the most abundant organism isolated, followed by *Pseudomonas spp.*

Figure 5 shows the result after evisceration in the slaughterhouse *E.coli* was the most abundant organism isolated followed by *Pseudomonas spp.*

Figure 6 shows the result in restaurant's meat product shawerma. *E.coli* was the most abundant organism isolated followed by *Pseudomonas spp.*

Figure 7 shows the result in restaurant's meat product burger. *E.coli* was the most abundant organism isolated followed by *Pseudomonas spp.*

shows the result of the total viable count in Butcher shop's raw meat.

E.coli was the most abundant organism isolated followed by *Pseudomonas spp.*in the second place and *Proteus spp*.

Table8. The Type of bacteria associated with *Salmonella* in the examined meat and meat products samples

samples	No of samples	Type and Percentage of isolate bacteria			
Slaughterhouse (after skinning)	50	*Salmonella* 1samples 2%	*E.coli spp* 25samples 50%	*Pseudomonas spp* 4samples 8%	*Proteus spp* -
Slaughterhouse after evisceration	50	4samples 8%	27samples 54%	4samples 8%	-
Shawerma	50	2samples 4%	25samples 50%	1sample 2%	-
Burger	50	0	15samples 30%	5samples 10%	-
Raw meat	50	10samples 20%	5samples 10%	10samples 20%	2samples 4%

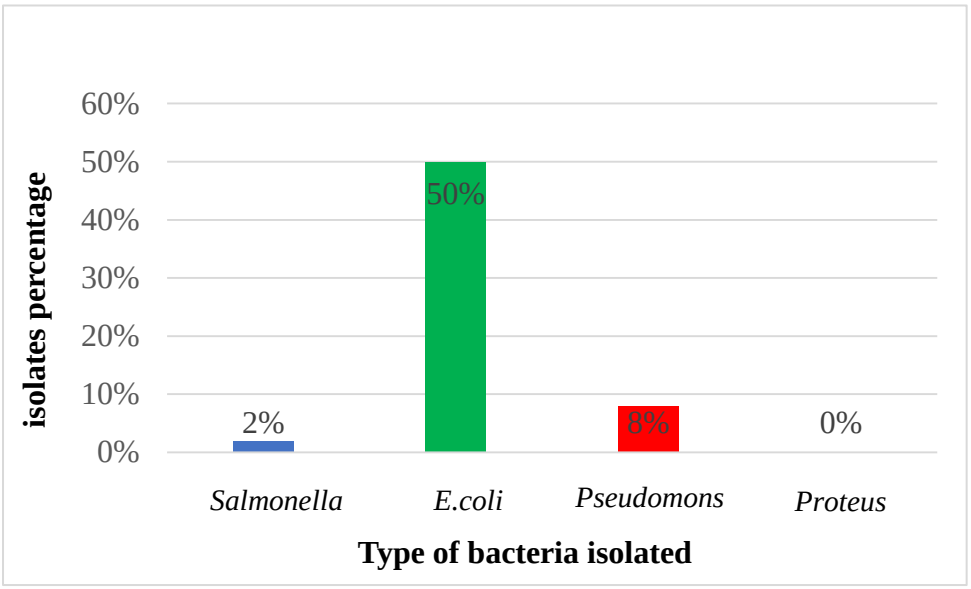

Figure 4. Percentage of bacteria isolated after skinning on Elkadarwo slaughterhouse.

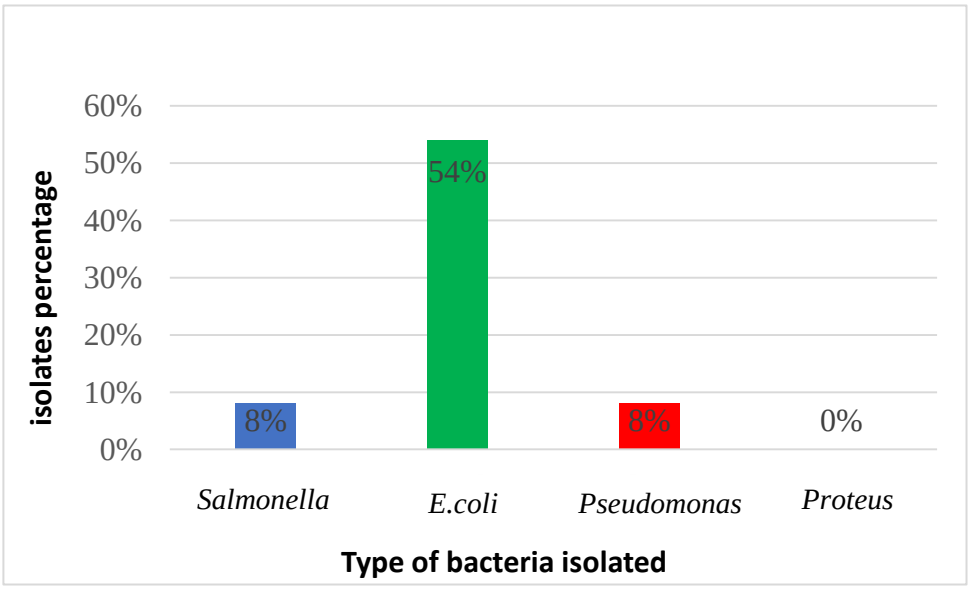

Figure 5. Percentage of the bacteria isolated after evisceration on Elkadarwo slaughterhouse.

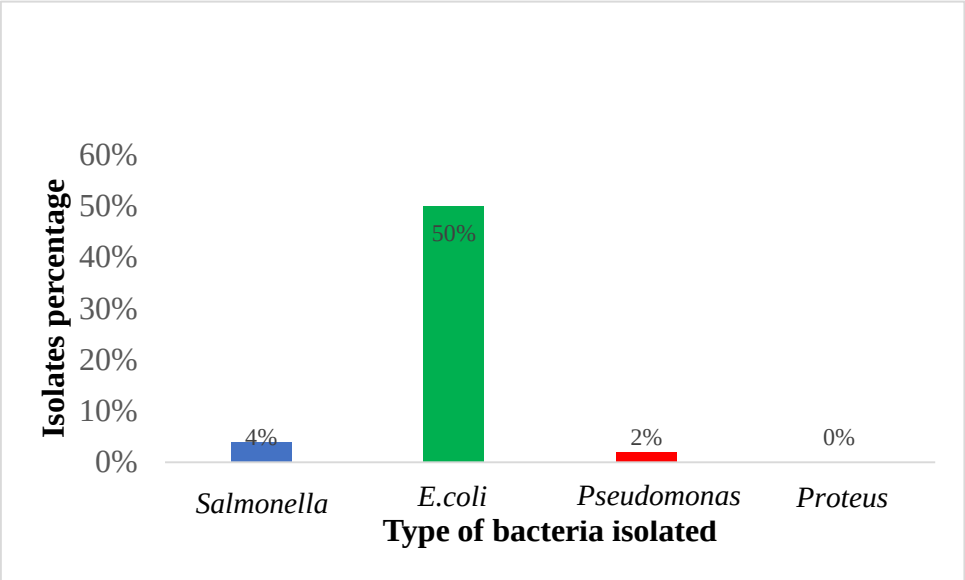

Figure 6. Percentage of bacteria isolated from restaurant meat product shawerma

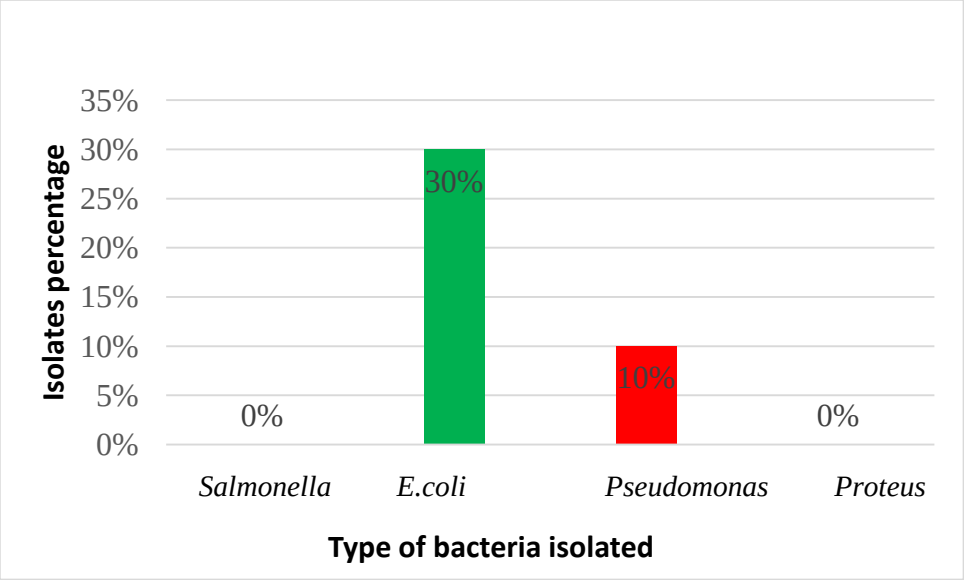

Figure7. Percentage of bacteria isolated from restaurant meat product burger.

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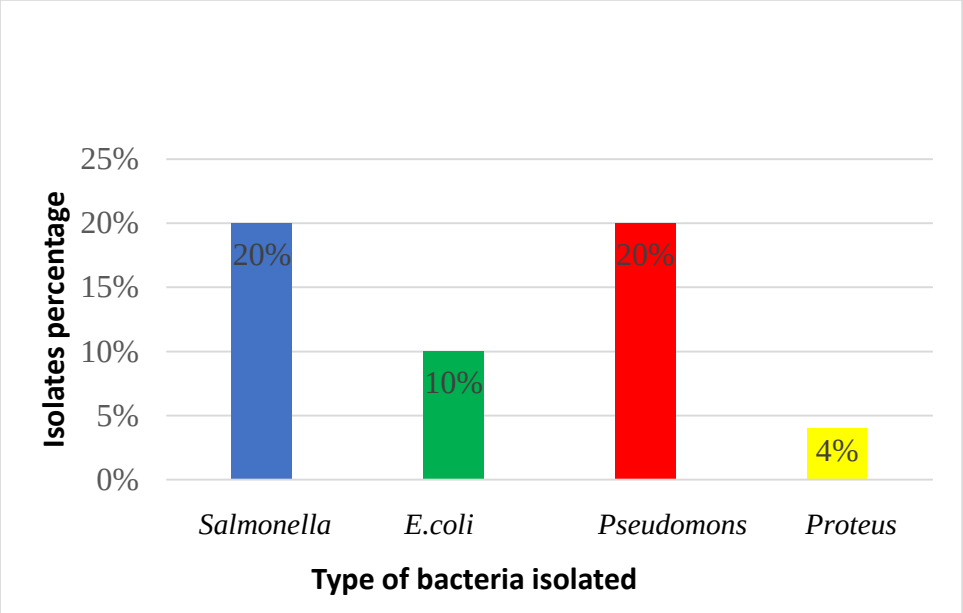

Figure 8. Percentage of bacteria isolated from Butcher shop' s raw meat.

3.5. PCR

3.5.1. PCR with primer pair Salm 3/ Salm 4

No amplification product was detected when control negative DNA was used as a template while the control positive gave a positive result. All tested DNA (N=17) produced amplicons of the expected size (□387bp) of the *Salm gene* (two from shawerma, one from cattle carcasses at the skinning stage, four from cattle carcasses at evisceration stage and ten from fresh meat at Butcher shops) (Table8). Figure 9 showed that the isolated *Salmonella* is *Salmonella universal.*

3.5.2. PCR with primer pair Styp F/ Styp R

The *Salmonella enterica.Typhimurium* amplicons expected size is (□433bp). This type of *salmonella* was not detected inAll tested DNA (N=17)product.

3.5.3. PCR with primer pair Styphi_F / Styphi_R

From the tested 17 DNA samples, the expected amplicons size(□738bp) of *Salmonella enterica ser. Typhi* was detected only in three isolates from fresh meat at Butcher shops (Table8).Figure 10 shows the PCR result of *Salmonella enterica ser. Typhi.*

3.5.4. PCR with primer pair Sdub F/ Sdub R

All tested DNA (N=17) produced amplicons of the expected size (□203bp) of the *Salmonella enterica ser. Dublin*(two from shawerma, one from cattle carcasses at the skinning stage, four from cattle carcasses at evisceration stage and ten from fresh meat at Butcher shops) (Table 8). Figure 11shows the PCR result of *Salmonella enterica ser. dublin.*

3.5.5. PCR with primer pair Sgal_F/ Sgal_R

The *Salmonella enterica ser. Gallinarum* amplicons of the expected size (□97bp) was not detected in All tested DNA (N=17) product.

Table 9.The tested DNA (N=17) produced amplicons of the expected size (□387bp) of the *Salm gene* from cattle meat

location	No of Salmonella	Type of *Salmonella*	Type of primers of PCR
Skinning	1	*dublin*	*S dubF/SdubR*
Evisceration	4	*dublin*	*S dubF/SdubR*
Shawerma	2	*typhi*	*S typhiF/StyphiR*
Burger	0	0	-
Butcher shop	10	7*dublin* and 3*typhi*	*7 SdubF/S dubR 3StyphiF/S typhiR*

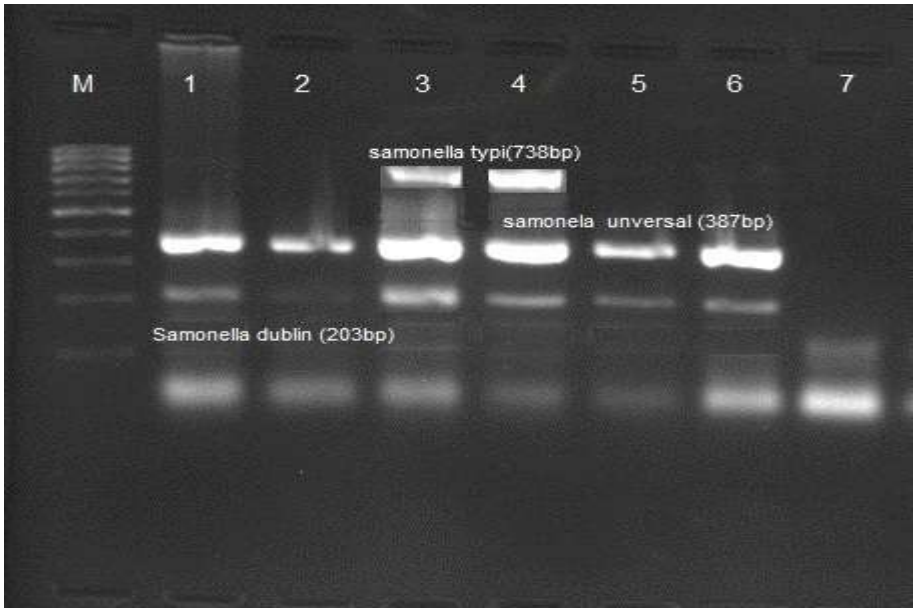

Figure 9 Positive swab samples from cattle carcasses by using agarose gel electrophoresis (Column M is the DNA marker lane)

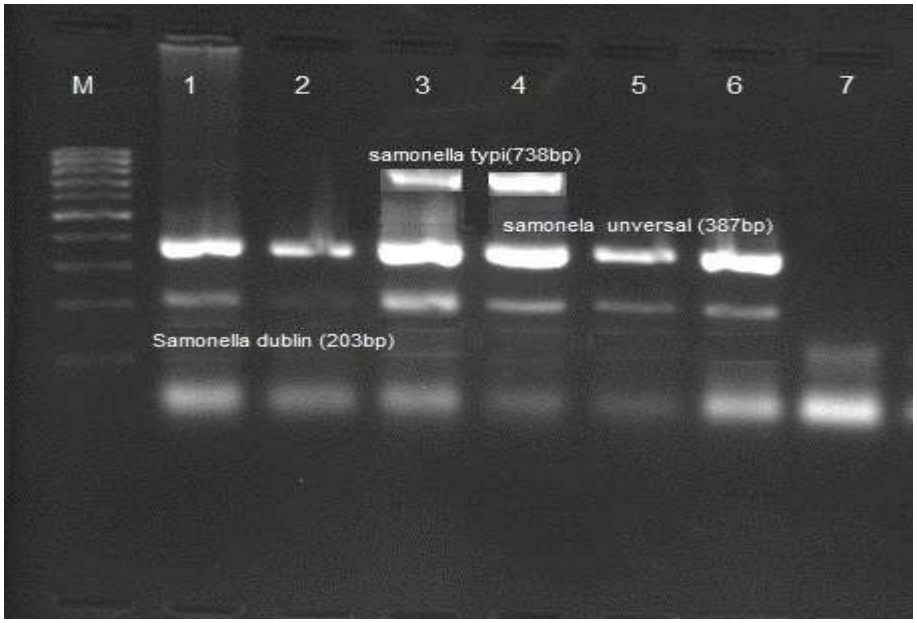

Figure 10 Agarose Gel Electrophoresis showing positive samples. Column M is the DNA marker lane.

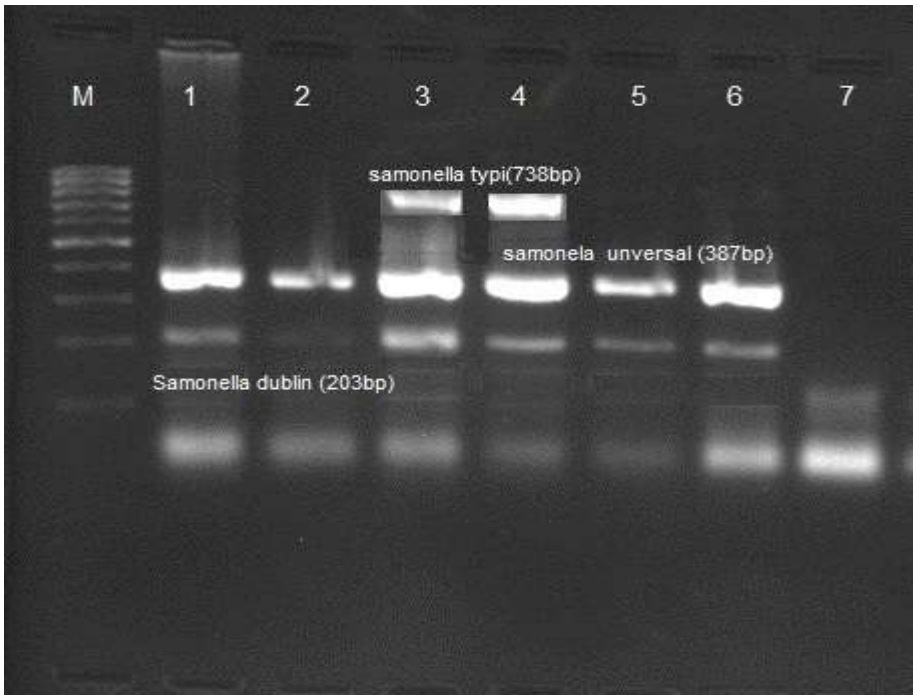

Figure 11 Agarose Gel Electrophoresis showing positive samples .Column M is the DNA marker lane.

Chapter Four

Discussion

Food borne salmonellosis is still today a serious public health issue: very common in poor developing countries, due to the bad general hygiene conditions and usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemyehu *et al* . , 2002 ; Arroyo and Arroyo , 1995). The real situation of salmonellosis in Sudan is needed more information .There are no criteria for routine microbiological monitoring plan, including analysis of raw meat and RTM cattle products for the presence of *Salmonella spp* in Sudan .In the present study for isolation and detection of *Salmonella* used of enrichment procedure followed by selective media are agreement with Vlaemynck *et al* (2000) and Beumer *et al* (2003).

The bacteriological examination of collected swabs revealed that the highest contamination levels recorded in the point of evisceration (4.29+- 1.34$\log_{10}$ CFU/cm^2) whereas the lowest contamination was recorded in the skinning stage (4.40+- 0.54 $\log_{10}$ CFU/cm^2) in all different operation at different sites on carcasses . The results of Amine *et al* (2013) revealed that after evisceration the bacterial count is high due to fecal contamination and the neck is most contaminated site. These findings are similar to those of Abdella *et al* (2009) who found the average TVCs after skinning ,evisceration and after washing in the abattoir were (5.5+0.89,6.0_+0.33 and 5.1+_0.4logCFU/$cm^{2,}$ respectively) , using conventional method for isolation . In this study TVCs after skinning is low (4.40+- 0.54 $\log_{10}$ CFU/cm^2) ,this agrees with Awatif (2012) who detected TVCs after skinning is low (2.85+-1.18 CFU/cm^2), and disagreed with Gill (1998) who reported bacterial contamination of meat during the different slaughtering operations . The highest level of TVCs after skinning was

from the neck at the slaughterhouse, (12×10^3 CFU/ML). This could probably be due to that the neck is the first part of the animal to be exposed to the ambient environment. This is disagreed with Getachew (2015) who detected higher prevalence of *Salmonella* were detected in the abdomen than the neck and hind limb. Awatif (2012) did not detect *Salmonella*, this is disagreed with this study which was detected Salmonella after evisceration . Out of the total 100 swab samples examined, 5(%5) were *Salmonella* positive and was agreement with D'Aoust (1989) who reported that the contamination rate of beef carcasses with *Salmonella* varies from 0.2-21.5 . Also disagree with Nyeleti *et al* (2000) reported a low prevalence of *Salmonella* in feces (2.2%) and mesenteric lymph nodes (4.2%) of slaughter cattle. On the contrary the same authors also reported a high prevalence of *Salmonella* in diaphragm (11.9%) and abdominal muscles (9.8%). This suggests the presence of severe cross-contamination during slaughtering process as a result of poor hygienic conditions during subsequent dressing operations. The other probable source of cross-contamination could be from *Salmonella* carrier slaughterhouse personnel (Molla *et al* ., 2003 ; Nyeleti *et al* .,2000) . Bacteriological examination of collected Ready to eat Meat samples revealed that the highest contamination levels recorded in the shawerma ($3.15+\_2..34\log_{10}$ CPU/cm^2) where as the lowest contamination was recorded in the burger ($2.3957+\_0.54\log_{10}CPU/cm^2$). Out of the total 50 samples examined, 2 (% 4) were *Salmonella* positive and was agreement with Tareq *et al* (2013) who detected the overall prevalence of *Salmonella serovars* in RTE products was 0.5%, with 0.8 and 0.2% in RTE chicken and RTE beef , respectively .And agrees with Abd El-Atty and Meshref (2007) who, detected *Salmonella* with a prevalence of 4% in sausages and 2% in spiced minced meat in Egypt. In this result contaminated Salmonella at butcher shops similar to Nyeleti *et al* (2000) from minced beef from supermarkets

in Addis Ababa and 9% prevalence from raw beef samples reported from butchers' shops in Awasa, Southern Ethiopia . *Salmonella* was detected by Getachew *et al* (2015) and the rate of 2.43% in the raw meat samples analyzed . Adesiyun and Oni (1989) detected contamination of meat by *Salmonella* occur at abattoir from the excretion of symptomless animal ,contaminated abattoir equipment, floor and personnel animals and the pathogen can gain access to meat at any stage during butchering. Many researchers such as (Tolba,1994 ; Duffy *et al* .,1999 ; Youssef *et al*., 1999 ; Fathi *et a*l, 2001) could not detect *Salmonella* species from samples of minced beef. They concluded that this negative result not indicates the absence of the bacteria, but this result may be due to low sensitivity and specificity of the method used in isolation. Also Vazgecer *et al* (2004) did not detect *Salmonella* in 72 chicken doner kebab samples in Turkey . These all studies are disagreed with this study which is finding contamination *salmonella* is higher in raw meat (20%).

In this study certain bacteria have been detected on the surfaces of the investigated Cattle carcasses, Cafeterias samples and butcher shop including:. *E coli*, *Salmonella spp*, *Pseudomonas spp* and *Proteus spp*. This agrees with Yassir *et al* (2015) who detected *E. coli, Salmonella* spp , *Pseudomonas spp, Shigella spp*, *Staphylococcus spp* and *Streptococcus spp* on the surfaces of the investigated carcasses. Phillips *et al* (2001) detected *E. coli* on 10.30% and in 5.10% of the investigated cattle carcasses and boneless beef samples as well as coagulase-positive, *staphylococci* on 24.30% of the carcasses and in 17.50% of the boneless beef. *Salmonella spp* on 0.20% of carcasses and in 0.10% of boneless beef were detected too. And disagreed with Tareq *et al* (2013) did not detected *E. coli* O157:H7 from any of these products.

In this study *Salmonella dublin* was the most frequently isolated serovar followed by S. typhi from raw meat samples. This is agrees with Molla and Alemyehu , (2002) they detected the predominant serotype *Salmonella dublin* , *S.braendurp* , *S.saintpaul* followed *S.typhimurium* , *S.anatum* and *S.entritidis* was detected from chicken ,camel and cattle meat. Also predominant *Salmonella* serotype reported by Molla *et al* (1999) from minced beef from supermarkets was *S. dublin*. And disagreed with Nyeleti *et al* (2000) reported S. *anatum* as a dominant serotype from minced beef samples collected from supermarkets in Addis Ababa.

Conclusion and Recommendation:

Conclusion

This study revealed that the highest contamination level was recorded in the evisceration stage in thigh region at Elkadrow slaughterhouse. Also at cafeterias the highest contamination level was recorded in Shawerma. The highest contamination level was recorded in butcher shop.

Recommendation

(1) An adequate water and disinfectant should be used to reduces bacterial contamination in Slaughterhouses.

(2)Using multiple primers for detection of multiple virulence associated genes of *Salmonella spp* by multiplex PCR is recommended

(3) Hazard Analysis and Critical Point (HACP)system should be applied in slaughterhouses

(4)Further studies are needed to determine source of *Salmonella Spp* and improve strategies to decrease the prevalence of *Salmonella Spp* in cattle meat .

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Appendix

Sterlization

Sterlization of equipments:

Sterilization techniques were done according to omer1986 .Biju and universal bottles were sterilized in autoclave at 15pound pressure for 15 minutes at 121 omer1986 .pertri dishes ,graduated pipette ,flask and test tubeswere sterilized in hot air oven at 160 for one hour.

Sterlization of culture, media and solution

, Deoxycholate agar , nutrient agar, and selenite F broth were sterilized in autoclave at 15pound for 15 minutes at 121°C

Sterilization by flame

Metal wire and loops were sterilized by flame .forceps treated by spirit and thensterilized by flaming

Disinfection

Before and during any work in laboratory bench were cleaned and disinfected byusing cotton dipped in ethyl alcohol solution 70%. Hands were also washed with soap and disinfectant.

Preparation of culture media

Nutrient agar:

This is composed of peptone 5.09g, beef extract 3.09g,sodium chloride8.09 gand agar 3g. the mixture was suspected in on liter of distilled water, then boiled to dissolve completely in a steamer and sterilized at 121°C for 15 minutes then cooled to 45-50 in water bath before dispended into sterile petri dishes(Barrow and felth man,.2003).

Selenite f broth

The medium is consist of pancreatic digest casein (5g) , lactose (4g) sodium selenite (4g) and sodium phosphate(10g) the mixer was dissolved in liter of distilled water by heating and sterilized at121cfor15 minutes

then cooled to 45-50°C in water bath before dispended into sterile petri dishes(leifson,E.,1936).

Deoxy cholateagar:

The medium is consist of lab lemco powder 5g,peptone 5g,latose 10g,sodium citrate 8.5g,sodium thiosulfate 5.4g,ferric citrate 1g,sodium deoxycholate 5g,neutral red 20g and agar 12g the mixer was dissolved in liter of distilled water by heating and sterilized at121cfor15 minutes then cooled to 45-50°C in water bath before dispended into sterile petri dishes(leifson,E.,1936).

Culturing and Purification of culture

After culturing the plates were incubated for 24 hours at 37^0C.purification was achieved by further sub culturing on nutrient agar and incubated at 37^oC for 24hrs.purification isolates were identified to their different features which included morphological, biochemical reaction to standard keys .(Barrow and Felthm,2003)

Gram's stain technique :

Films were made from purified culture on clean glass slides, then air dried and fixed by heat .The stain used was crystal violet (2min) and lugol's iodine (30sec) after washing by tap water .The slides were decolorized by alcohol and washed ,then counter stained with dilute carbolfuchsin (15sec).Examination of the slide after washing and drying under the microscope in oil immersion lens. Positive organism identified y blue coloration and negative organism showed red coloration.