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**OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* IN
FECAL AND CARCASS SWAB SAMPLES OF SMALL RUMINANTS AT ADDIS
ABABA LIVESTOCK MARKET AND ABATTOIR**

MVSc Thesis

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Bishoftu, Ethiopia

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis
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Veterinary Science in Veterinary Microbiology

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ABBERRVIATIONS

ANOVA – Analysis of Variance

API 20E – Analytical Profile Index 20 Enterobacteriaceae

BPW - Buffered Peptone Water

CLSI – Clinical and Laboratory Standard Institute

CSA - Central Statistical Agency

GIT – Gastro Intestinal Tract

HACCP - Hazard Analysis Critical Control point

ISO - International Organization for Standardization

LIA - Lysine Iron Agar

MR - Methyl Red

OIE – Office International des Épizooties

PCR – Polymerase Chain Reaction

R/Y/ H_2S^+ – Red/Yellow/ Hydrogen Sulfide Positive

RV broth - Rappaport-Vassilliadis broth

SMT - Sulphamethoxazole and Trimethopirim

TSI - Triple sugar iron

VP - VogesProskauer

ABSTRACT

A cross sectional study was undertaken to investigate the occurrence and antimicrobial susceptibility of *Salmonella* from small ruminants brought for sale at different markets of Addis Ababa and those slaughtered at abattoir. Different sample types (fecal, carcass swab, meat and feed) were collected and cultured for *Salmonella* using standard procedure by pre-enriching in Buffered Peptone Water and enriching in Rappaport-Vassiliadis enrichment Broth (RVB) and Tetrathionate broth (TTB). It was then streaked from both RV and TTB to Xylose Lysine Deoxycholate (XLD). Presumptive *Salmonella* colonies were confirmed by various biochemical tests and *Salmonella* Genus Specific Polymerase Chain Reaction (PCR). *Salmonella* isolates were tested for their susceptibility to 17 antimicrobials. The overall occurrence of *Salmonella* in sheep and goat fecal samples at Addis Ababa livestock market was 4.08% (12/294) (95% CI: 1.8-6.4%) and its occurrence in carcass swab samples collected from Addis Ababa public abattoir was 0.85% (1/117) (95% CI: 0.8-2.5%). There was no statistically significant difference in occurrence of *Salmonella* in sheep and goats ($p > 0.05$). But nearly a significant difference ($p=0.052$) for the occurrence of *Salmonella* between the carcass swab of goats (3.12%) and sheep (0.0%) was seen. All the 13 *Salmonella* isolates were susceptible to the four drugs (amikacin, cefoxitin, chloroamphenicol and nalidixic acid) and 92.30% of the isolates were susceptible to Ceftriaxone and Nitrofurantoin. Resistance to streptomycin was observed in 84.62% of the *Salmonella* isolates followed by gentamicin (67%), tetracycline (54%) and trimethoprim (53%). Intermediate resistance was seen towards cephalothin, neomycin and ciprofloxacin in 23.08%, 15.38% and 15.38% of the isolates, respectively. All of the 13 *Salmonella* isolates were resistant to at least one of the 17 antimicrobials tested. Seven (53.85%) were resistant to 4-8 antimicrobials. In conclusion, *Salmonella* is more common in fecal samples of sheep than the carcass swab and most of the isolates were multidrug resistant. To hamper the burden of *Salmonella* infection and contamination in live animals and animal products, it is critical that risk reduction strategies should be implemented throughout the food chain.

Keywords: *Salmonella* Sheep, Goat, Feces, Carcass, Antimicrobial,

1. INTRODUCTION

Salmonella are facultative anaerobic Gram-negative rods within the family of *Enterobacteriaceae*. They can grow at an optimum temperature of 37°C, and can be killed by temperature of 55°C (Anderson, 1996). It is closely related to the genus *Escherichia* and is found worldwide in cold-blooded and warm blooded animals as well as in non-living habitats (Yan *et al.*, 2003). Most *salmonella* catabolize a variety of carbohydrates with the exception of *Salmonella* serotype Typhi which only produces acid, whereas lactose and sucrose are not fermented by the majority of *Salmonella* (Barbara *et al.*, 2000; Rao, 2004).

Salmonellosis is economically important infectious disease of animals (Radostits *et al.*, 1994; OÍÉ, 2000). *Salmonella* infection in animals occur mostly through the ingestion of contaminated feed and water or through contact with the contaminated excretions of latently infected animals; shedders always playing a major role in the dissemination of the organisms (Seifert, 1996). Though primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry (Wray and Davies, 2003).

Salmonella are readily transferred from animal to animal, animal to humans, and human to human by direct or indirect pathways (Clarke and Gyles, 1993). Food borne *Salmonella* typically causes acute gastroenteritis and may cause a more septicemic disease usually in very young, the elderly and immune compromised subject (Enyew *et al.*, 2016). *S. Infantis*, *S. Butantan*, *S. Braenderup*, *S. Kingabwa*, *S. Zanzibar*, *S. Kottbus*, *S. Anatum*, *S. Typhimurium* and *S. Hadar*, *S. Kottbus*, were the most common serotypes isolated from samples originated from apparently healthy slaughtered sheep and goats in

Addis Ababa supermarkets (Ejeta *et al.* (2004), and Elfora Debre Zeit abattoir (Woldemariam ,2003).

The use of antimicrobials for prophylaxis in food producing animals has been a great concern, because it is believed to be an important factor in the emergence of strains resistant to certain antimicrobials. However, the exact causes of the emergence of these resistant strains have yet to be pinpointed (Threlfall *et al.*, 2000). Streptomycin, chloroamphenicol, ampicillin, ciprofloxacin, gentamycin, cefotaxime, amoxicillin, kanamycin, nalidixic acid, ciprofloxacin are commonly used drug in Ethiopia (Fufa *et al*, 2017). Study by Zelalem *et al.*,(2011) and Fluckey *et al.*, (2006) states use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risk associated with consumption of contaminated meat products.

Ejeta *et al.* (2004) reported a 14.1% *Salmonella* contamination rate of mutton collected from Addis Ababa supermarkets, and other studies on prevalence of *Salmonella* in various food animals and animal products in Ethiopia. Even if there are different studies carried out in Addis Ababa on sheep and goats and other food animals, little information is available on prevalence and antimicrobial resistance of *Salmonella* in small ruminants at Addis Ababa market and ready for sale and slaughtering. Therefore the objectives of this study are

- To determine the occurrence of *Salmonella* in feces of small ruminants brought from various regions of the country to Addis Ababa markets and also in meat and carcass swab samples collected from Addis Ababa abattoir and butcher shops.
- To determine the antimicrobial resistance of isolated *Salmonella*.

2. LITERATURE REVIEW

Salmonella is a rod-shaped, Gram-negative, oxidase negative, non-spore forming, facultative anaerobic and predominantly motile bacteria. The optimum temperature for growth of *Salmonella* is 37°C but cannot survive temperature higher than 70°C. *Salmonella* produces hydrogen sulfide when grown on agar media; black precipitate being used as the indicator of hydrogen sulfide production (Mastroeni and Maskell, 2006). *Salmonella* ferments glucose, manitol, arabinose, maltose, dulcitol and sorbitol producing acid and gas except *S. Typhi* and *S. Gallinarum* which produce only acid and no gas. They are indole negative, urease negative and citrate positive except *S. Typhi* and *S. Paratyphi A* which are citrate negative as they need tryptophan as growth factor. (Kumar, 2012).

Most of the members of this genus are motile by peritrichous flagella except *S. Pullorum* and *S. Gallinarum*. *Salmonellae* are frequently facultative intracellular parasites. *Salmonella* are non-capsulated except *S. Typhi*, *S. Paratyphi C* and some strains of *S. Dublin*. These bacteria can resist dehydration for a very long time (Brenner et al., 2000).

Although primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry (Wray and Davies, 2003).

2.1. The Genus *Salmonella*

2.1.1. Taxonomy and Classification

Salmonella are Gram negative bacilli which can grow both aerobically and an aerobically (facultative anaerobic) belonging to the family *Enterobacteriaceae* and includes a large number of serotypes affecting human beings as well as mammals, which are antigenically related to one another (Anderson, 1996).

There are two species of *Salmonella*: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* consists of six subspecies (Brenner *et al* 1998 and Popoff *et al* 2001): I, *Salmonella enterica* subsp. *enterica*; II, *Salmonella enterica* subsp. *salamae*; IIIa, *Salmonella enterica* subsp. *arizonae*; IIIb, *Salmonella enterica* subsp. *diarizonae*; IV, *Salmonella enterica* subsp. *houteanae*; and VI, *Salmonella enterica* subsp. *indica*.

In subspecies I, serotypes (or serovars) are designated by a name usually indicative of the associated diseases, their geographic origins where it was first isolated, or their usual habitats. In the remaining subspecies as well as those of *S. bongore*, antigenic formulae determined according to the Kauffmann-White scheme (Popoff and Le Minor, 2001 and Jay, 2000) are used for those unnamed serotypes. Some members of these subspecies may have been named before 1966 and thus their names are retained and cited as those in the subspecies I.

To avoid confusion between serotypes and species, the serotype name is not italicized and starts with a capital letter. When cited at the first time in a report, the genus name is given followed by the word “serotype” (or the abbreviation “ser.”) and then the serotype name, e.g., *Salmonella* serotype or ser. Choleraesuis, and *Salmonella* serotype or ser. Typhi. Afterward the name may be shortened with the genus name followed directly by the serotype name, e.g., *Salmonella* Choleraesuis or *S. Choleraesuis*, and *Salmonella* Typhi or *S. Typhi* (Popoff *et al*, 2004). Because the type of species name, *enterica*, has

not been approved before 2005, serotype names are used directly after the genus name without the mention of the species.

For those designated by their antigenic formulae, the subspecies name is written in Roman letters (not italicized) followed by their antigenic formulae, including O(somatic) antigens, H (flagellar) antigens (phase 1), and H antigens (phase 2, if present). A colon is used in between each antigen, e.g., *Salmonella* serotype II39:z10: z₆. For serotypes in *Salmonella bongori* (previously belongs to subgenous V), V is still used for consistency, e.g., *S. V* 13,22:z35:- (Popoff MY *et al*, 2004).

The 2006 ASM (In publications of the American Society for Microbiology (ASM)) Instruction to Authors indicated that, for the species, “*Salmonella enterica*” is used at the first time, and “*S. enterica*” thereafter; for the subspecies, “*Salmonella enterica* subsp. *arizonae*” is used at first, and “*S. enterica* subsp. *arizonae*” thereafter. Serotype names should be in Roman type with the first letter capitalized, e.g., *Salmonella enterica* serotype Typhimurium. After the first use, the serotype may be used without a species name, e.g., *Salmonella* Typhimurium.

Based on their association with human and animal hosts *Salmonella* can be classified into three main groups. The first group comprises *Salmonella* Typhi and Paratyphi A and C, which infect only man and *S. Gallinarum* which infects only poultry. They spread either directly or indirectly (via food and water) from person to person or from bird to bird (WHO, 1988; Radostits *et al.*, 1994). They are also referred as host restricted *Salmonella* serotypes. The second group includes serovars that are host adapted for particular species of vertebrates, example *S. Dublin* in cattle, *S. Abortusequi* in horse, *S. Abortusovis* in sheep and *S. Choleraesuis* and *S. Typhisuis* in swine. Some of these are also pathogenic to man (especially *S. Dublin* and *S. Choleraesuis*). The third group contains the majority of other *Salmonella* serovars with no particular host preference that infect both animals and man (Jay, 2000).

2.2. Epidemiology

Studies conducted in Ethiopia indicated the presence of *Salmonella* in animals, humans, foodstuffs and environment. Salmonellae were isolated from human, chicken, cattle, camels, sheep, goats and food (Nyeletiet *et al.*, 2000; Tibaijuku, 2003; Woldemariam, 2003). In a study conducted by Pegram *et al.* (1981), 27 *Salmonella* serotypes were isolated from samples of animal origin in different parts of the country. In sheep and goats, Woldemariam (2003) reported a 6.4% prevalence of salmonellosis at ElforaDebreZeit abattoir. According to Woldemariam (2003), out of the 642 samples from sheep (n=282) and goats (n=360) examined for *Salmonella*, 33 *Salmonella* isolates consisting of 9 different serotypes were identified. Ten (3.5%) of apparently healthy slaughtered sheep and 23 (6.4%) of goat samples were found to be contaminated with *Salmonella*. The serotype identified were *Salmonella* Infantis (45%), *Salmonella* Butantan (24.2%), *Salmonella* Braenderup, *Salmonella* Kingabwa (each 6%), *Salmonella* Zanzibar, *Salmonella* Kottbus, *Salmonella* Anatum, *Salmonella* Typhimurium and *Salmonella* Hadar (each 3%). Ejeta *et al.* (2004) also isolated five different serotypes (*Salmonella* Infantis (25%), *Salmonella* Braenderup (42%), *Salmonella* Anatum (8.3%), *Salmonella* Bovismorbificans(17%), and S. I: 47, z₄, z₂₃) from mutton. The habit in this country to consume, raw meat as a traditional food poses a high risk of acquiring Salmonellosis as well as other zoonotic diseases (Mollaet *et al.*, 1999a; Mollaet *et al.*, 2003a).

The prevalence commonly precipitates by stressful situations such as sudden deprivation of feed, transportation, drought, crowding, parturition, surgery, and administration of certain drugs, including oral antibiotics. Greater susceptibility in the very young may be the result of high gastric pH, absence of a stable intestinal flora, and limited immunity (wray and Linklater, 2000).

2.3. Source of *Salmonella* infection, ways of transmission and pathogenesis

Salmonella may survive for long periods in the environment; it is the carrier state that provides the major source of infection for animals and human (Clarke and Gyles, 1993; Radostitset *al.*, 1994). Infected animals are the source of the organisms which they excrete and infect other animals directly or indirectly by contamination of the environment, primarily feed and water supplies. Ingestion of infected food and water or contact with contaminated excretions is the main ways of infection (Baird-parker, 1990; Radostitset *al.*, 1994).

After ingestion, the organism colonizes the digestive tract and invades and multiplies in enterocytes and tonsillar lymphoid tissue. Penetration of bacteria into the lamina propria contributes to gut damage and diarrhea. The cellular infection results in activation of a host alarm process through signalling molecules as a result of the detection of bacterial surface proteins, which in turn induces a strong inflammatory response that generally is able to restrict the bacteria to the intestine. Some serotypes also become localized in the reproductive tract. Serotypes that are able to cause typhoid can modulate the initial host response and suppress the inflammatory response. As infection progresses, a true septicemia may follow, with subsequent localization in brain and meninges, pregnant uterus, joints and distal aspects of the limbs, and tips of the ears and tails, which can result in meningoencephalitis, abortion, osteitis, and dry gangrene of the feet, tail, or ears respectively. The organism also frequently localizes in the gallbladder and mesenteric lymph nodes, and survivors intermittently shed the organism in the feces. Infection may also persist in lymph nodes or tonsils, with no salmonellae in the feces. Latent carriers may begin shedding the organism or even develop clinical disease under stress (kemal, 2014).

2.4. Bacteriological examinations

Samples for bacteriological tests are collected as aseptically as possible and before any antibiotic treatment has commenced. Samples are collected preferably during the acute phase of the disease or as soon as possible after death (OIÉ, 2000).

In the live animal, confirmation of clinical salmonellosis is performed by the culture of rectal swabs or preferably, freshly voided faeces. The organisms may be isolated by blood culture and some time from the milk. At necropsy, the isolation of *Salmonella* from tissues and intestinal contents usually presents few problems, but care must be taken in the interpretation of the findings. The isolation of *Salmonella* should be correlated with clinical signs and pathological lesions in order to determine the significance of the isolation (Wray and Davis, 2000).

The isolation and subsequent identification of *Salmonella* is dependent not only on the quality of samples but also on the culture medium and growth characteristics of the serovars, particularly those adapted to the host species (OIÉ, 2000).

2.5. Conventional cultural isolation techniques

According to the International Organization for Standardization (ISO 6579, 1998) it is customary to use three stage processes: pre-enrichment, selective enrichment and selective plating to isolate *Salmonella*.

Pre-enrichment: Pre-enrichment allows the resuscitation and multiplication of sub-lethally damaged *Salmonella* cells (Blackburn, 1993). Non-selective media such as buffered peptone water and lactose broth are most widely used for resuscitation; buffered peptone water being recommended for routine purposes. The need for resuscitation is

now widely accepted for all types of samples and not merely those which have been dried or frozen (Varnam and Evans, 1991).

Selective enrichment: Selective enrichment helps to increase the ratio of *Salmonella* to competitor organisms. Many types of inhibitors have been proposed for the selective enrichment of *Salmonella*, the most widely used of which are bile, tetrathionate, selenite and dyes such as brilliant green and malachite green. Various formulations of selenite and tetrathionate broths have been widely used, although in recent years there has been increasing use of the malachite green based Rappaport-Vassiliadis (RV) broth (Varnam and Evans, 1991; Blackburn, 1993).

Selective plating: Plating on selective agar media enables the recognition of *Salmonella* colonies while suppressing the growth of the background microflora. A wide range of media has been devised for selective plating. Selective plating media for *Salmonella* all contain a diagnostic system to permit differentiation of the organisms from non-*Salmonella*. This is commonly based on the inability of most salmonella serotypes to ferment lactose and, in some cases, other carbohydrates such as sucrose and salicin. Bile containing media often employ a second diagnostic system based on the ability of *Salmonella* to produce hydrogen sulphide. Where competition from other bacteria is insignificant, a general-purpose medium such as MacConkey agars may be used. In many cases, greater selectivity is required and it is necessary to use a medium devised specially for *Salmonella*, such as brilliant green agar, *Salmonella-Shigella* agar, xylose lysine desoxycholate agar and Rambach agar (Blackburn, 1993; Quinn *et al.*, 1994).

2.6. Biochemical tests

Colony characteristic for *Salmonella* on the selective/indicator media are inoculated singly onto a triple sugar iron (TSI) agar slope and lysine decarboxylase broth. The typical reactions for *Salmonella* in TSI agar is a red (alkaline) slant, yellow (acid) butt and superimposed (black) H_2S production (R/Y/ H_2S^+). *Salmonellae* typically produce an alkaline (purple) reaction in the tube of lysine decarboxylase broth. If the reaction in TSI agar and lysine decarboxylase broth is equivocal, further biochemical tests such as indole (negative), methylred (positive), citrate (positive), urease (negative), Voges-Proskauer test (negative) and others should be carried out or an identification system used such as API 20E (analytab products) or Enterotubes (RoheDiagnostica) (Doyle and Cliver, 1990; Quinn *et al.*, 1994).

2.7. PCR Assay

PCR (polymerase chain reaction) is a method to analyze a short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR uses a small amount of template DNA, two primers that flank the target sequence, nucleotides, and thermostable DNA polymerase to amplify a specific region of DNA, thus creating a large amount of DNA from a very small sample. PCR is extremely sensitive in that only trace amounts of the template DNA that is the sequence to be amplified are necessary in the reaction. The cycles include three different steps: denaturation of the template DNA into single strands at about 90°C, a moderate temperature (around 50° to 60°C) to anneal the primers to the target sequence, and an extension step in which DNA polymerase elongates a second strand of DNA complementary to each strand of the target (Clark P. D, and Pazdernik J. N, 2013).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Addis Ababa, the capital city of Ethiopia. The city lies at 9°1' North latitude, 38°44' East longitude and altitude range of 2000 - 3000 meters above sea level/masl/. Its annual rain fall ranges from 750-1500 millimeters and the average daily temperature is about 16°C (Hunderra *et al.*, 2012). Specifically, four sheep and goats' market (Akaki-Kality, Nock_18, Kera, Kara and Yeka) areas within Addis Ababa and Addis Ababa municipal abattoir (during antemortem examination) were used for sample collection from live animals. Abattoir and butcher shops in the respective market areas were also used for carcass swabs and meat sample collections. The laboratory work for the samples was conducted in Microbiology laboratory of Aklilu Lemma Institute of Pathobiology of Addis Ababa University.

3.2. Study design and sample size determination

A cross-sectional study design was conducted to investigate occurrence of *Salmonella* in feces of small ruminants sold in Addis Ababa and carcass swab and meat samples from slaughtered small ruminants. The sample size required for the study was determined depending on the expected prevalence of salmonellosis and the desired absolute precision as per the method of Thrusfield (1995).

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Using a 95% confidence level, 5% absolute precision and 7.4% expected prevalence for goats and sheep (Tigist, 2017 4.17%); a sample size of 61.4 was expected. Even though, the desired sample size is as indicated here; for sake of better representative sample size 234 sheep and 60 goats were considered for fecal sample during the study period. Moreover, 117 carcass swab samples, 10 pieces of meat samples and 9 feed samples were also sampled purposely.

3.2.1. Study population, study animals and sampling procedure

Study population were all sheep and goat population sold on open air markets in Addis Ababa at various places. A total of 294 randomly selected animals for fecal sample $n=234$ for different sheep breeds and $n=60$ for goats brought from various regions of the country to Addis Ababa live stock market Akaki-Kality, Nock_18, Kara and Yeka areas and Addis Ababa abattoir individually or in flocks by vehicle to be sold and slaughtered were used as study animal. Randomly selected carcass swabs from, ramp, sternum, brisket and neck area of sheep ($n=85$) and goats ($n=32$) slaughtered in Addis Ababa abattoir by using gauze were used as additional sample. More over feed samples ($n=9$) from the watering and feeding troughs and piece of meat samples ($n=10$) from butcher shops were also used for the study.

On each sampling day, from each animal market area, once oral consent from the animal owner was obtained (species, breed, sex and body conditions were concerned), the sheep types in Ethiopia were classified into four major groups based on their physical characteristics: short fat-tailed, long fat-tailed, thin-tailed and fat-rumped sheep. Based on DNA differences, Ethiopian sheep types have been classified into nine genetically distinct breeds (Table 2; Gizaw *et al.*, 2008). Scoring sheep and goats is done using a BCS ranging from 1.0 to 5.0, with 0.5 increments. according to previously described procedures (Girma *et al.*, 2007; John, 2011). An animal of BCS 1.0 is extremely thin

with no fat reserves and a BCS of 5.0 is a very over-conditioned (obese) animal. In most cases, healthy sheep and goats should have a BCS of 2.0 to 3.5. A BCS below 2.0 indicates a management or health problem. A BCS of 4.5 or 5 is almost never observed under normal management conditions. To assign a BCS, one must touch and feel the animal. In sheep, the lumbar region is the principal site for BCS determination while in goats the rib cage and sternum also play a role. Before scoring the animal should be standing in a relaxed position. It should not be tense, crushed by other animals or held in a crush. If the animal is tense it is not possible to feel the short ribs and get an accurate condition score. Table 1 shows body conditions scoring guidelines used in this study.

Table 1. Scales for body condition scoring

Condition	Score	Lumbar region	Rib cage	Sternum
Starving	0	Extremely emaciated and on the point of death. It is not possible to detect any muscle or fatty tissue between the skin and the bone.	Skin is sunken between visible ribs.	There is no sternal fat.
Very thin	1	The spinous processes are prominent and sharp. The transverse processes are also sharp, the fingers pass easily under the ends, and it is possible to feel between each process. The eye muscle areas are shallow with no fat cover.	Ribs are clearly visible.	Sternal fat is easily grasped and moved from side to side.
Thin	2	The spinous processes feel prominent but smooth, and individual processes can be felt only as fine corrugations. The transverse processes are smooth and rounded, and it is possible to pass the fingers under the ends with a little pressure. The eye muscle areas are of moderate depth, but have little fat cover.	Some ribs can be seen. There is a small amount of fat cover. Ribs are still felt.	Sternal fat is wider and thicker but can still be grasped and moved slightly from

				side to side.
Moderate	3	The spinous processes are detected only as small elevations; they are smooth and rounded and individual bones can be felt only with pressure. The transverse processes are smooth and well covered, and firm pressure is required to feel over the ends. The eye muscle areas are full, and have a moderate degree of fat cover.	Ribs are barely seen; an even layer of fat covers them. Spaces between ribs are felt using pressure.	Sternal fat is wide and thick. It can still be grasped but has very little movement.
Fat	4	The spinous processes can just be detected with pressure as a hard line between the fat covered eye muscle areas. The ends of the transverse processes cannot be felt. The eye muscle areas are full, and have a thick covering of fat.	Ribs are not seen.	Sternal fat is difficult to grasp and cannot be moved from side to side.
Very fat	5	The spinous processes can't be detected even with firm pressure, and there is a depression between the layers of fat in the position where the spinous processes would normally be felt. The transverse processes cannot be detected. The eye muscle areas are very full with thick fat cover. There may be large deposits of fat over the rump and tail.	Ribs are not visible and are covered with excessive fat.	Sternal fat extends and covers the sternum. It cannot be grasped

Adopted from Girma *et al.*, (2007)

Sheep and goats to be involved in the study were randomly selected for sampling. From each selected animal, about 10gms of feces was collected separately in to sterile zippered plastic bags by using examination glove directly from the rectum. Moreover, carcass swabs and meat samples from Addis Ababa abattoir and butcher shops and feed samples

from feeding and watering trough were collected in aseptic manner as additional sample. Then each sample was appropriately labeled and accompanied by necessary identifying information, which include date of sampling, type of sample, species of animal, and identification code. Samples were then transported with ice box directly to Microbiology Laboratory, Aklilu Lemma Institute of Pathobiology, Addis Ababa University and processed for *Salmonella* isolation.

Table 2. Major groups, breeds and types of sheep in Ethiopia

Major group	Breed	Sheep type	Tail type/shape	Fiber type
Short-fat-tailed	Simien	Simien	Fatty and short	Wool/fleece
	Short fat-tailed	Sekota, Farta, Tikur, Wollo, Menz	Fatty and short	Wool/fleece
	Washera	Washera	Fatty and short	Short hair
Long-fat-tailed	Horro	Horro	Fatty and long	Short hair
	Arsi-Bale, Adilo(Wolaita)	Arsi-Bale, Adilo	Fatty and long	Short hair
	Bonga	Bonga	Fatty and long	Short hair
Fat-rumped sheep	Afar(Adal)	Afar	Fat rump with fat tail	Short hair
	Black head Somali(Wanke)	Black head Somali	Fat rump/tiny tail	Short hair
Thin-tailed sheep	Gumz	Gumz	Thin and long	Short hair

Source: Gizaw, S., *et al.*, (2008)

3.3. Isolation and identification of *Salmonella*

3.3.1 Isolation of *Salmonella*

Fresh fecal samples, (10 g of fecal pellets of sheep and goats was broken in to pieces) pre-enriched in 90 ml of buffered peptone water (BPW) (Becton Dickinson, Sparks, MD) and incubated over night at 37°C. Similarly 10 gram of meat sample was pre-enriched in 90 ml of BPW; carcass swab samples collected in test tubes containing 10 ml of BPW was incubated at 37°C. Hundred µl of pre-enriched suspension was added into 9.9 ml of Rappaport-Vassiliadis enrichment Broth (RVB) (Oxoid, USA) and incubated at 42°C for 24 h. At the same time, 1ml of suspension was also transferred to 10 ml of Tetrathionate broth (TTB) (Oxoid, USA) and incubated for 24 h at 37°C. It was then streaked from both RV and TTB to Xylose Lysine Deoxycholate (XLD) (Oxoid CM0469, Basingstoke, England) agar selective media and the plates were incubated at 37°C for 24 to 48 h. Presumptive *Salmonella* colonies having a slightly transparent zone of reddish color and a black center, was subcultured on nutrient agar (Oxoid CM0003, Basingstoke, England) and various biochemical tests were conducted.

3.3.2 Biochemical tests

Suspected *Salmonella* colonies were tested biochemically according to ISO (1998) using triple sugar iron agar (TSI) (Oxoid CM0277, Basingstoke, England), urea agar (Oxoid CM53, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England)(Hendriksen., 2002) as described here below:

Triple sugar iron (TSI) agar test: The TSI agar tube was inoculated towards the bottom at the middle of the agar for 5mm with a straight inoculating wire and on the withdrawal of the straight wire the entire slant will be streaked and incubated at 37°C for 16 to 24 hours. The typical reactions for most *salmonellae* were seen as red slant, yellow butt with

gas production and superimposed black color with hydrogen sulphide production (Quinn *et al.*, 1994; ISO, 1998).

Citrate utilization test: Simon citrate agar slope surface was streaked with the suspected *Salmonella* colonies and incubated at 37°C for 18 to 24 hours. Typical reactions for citrate utilization were declared by the change of the medium from green to blue color (Quinn *et al.*, 1994).

Lysine decarboxylase test: The lysine decarboxylase broth was inoculated with the suspected *Salmonella* colonies just below the surface of the liquid medium and was incubated for 18 to 24 hours at 37°C. As it was described by Quinn *et al.* (1994) and ISO (1998), the purple color after incubation was considered as positive reaction for *Salmonella* and a yellow color as negative.

Urease test: inoculum of the presumptive *Salmonella* colonies was inoculated into the urea broth tube and incubated at the 37°C for about 48 hours. As it was described by Quinn *et al.* (1994), yellow coloration of the broth because of inability of *Salmonella* organism to split urea considered as negative and a red coloration as positive.

3.3.3 Polymerase Chain Reaction (PCR) for confirmation test

Those colonies with typical *Salmonella* biochemical properties were then further confirmed by genus specific PCR according to Cohen *et al* (1993). For isolation of *Salmonella* genomic DNA, each isolates were cultured on tryptone soya agar plate and incubated for 24 hours at 37 °C. Then pure colony was picked and suspended in 100 µl nuclease-free water (UltraPure DNase/RNase-Free Distilled Water, Thermo Fisher, USA) in PCR tubes. Extraction of DNA was performed by boiling the suspension for 5 min at 95°C in thermo cycler, centrifuged at 6,000 rpm for 5min. And an aliquot (1 µl) of the supernatant was used as template for PCR amplification.

Reference strain of *S. Typhimurium* (ATCC 14028) was used as a positive control during biochemical analysis and PCR.

PCR Reaction mix (20 µl) consisting 18 µl nuclease-free water, 1 µl of the template DNA, 0.5 µl of each of primers (reverse and forward) was prepared and added into lyophilized PCR premix (AccuPowerTaq PCR preMixBioneer, Korea) tubes which contains 1U Taq DNA polymerase, 250 µM dNTPs (dATP, dCTP, dGTP, dTTP), 1x reaction buffer with 1.5 mM MgCl₂, trace amount of tracking dye and patented stabilizer which were already aliquoted in PCR tubes and dissolved and spun down. The mixture was then amplified using thermal cycler with an initial denaturation (4 min at 95°C) followed by 30 cycles of denaturation (30 sec at 95°C), annealing (30 sec at 60°C) and elongation (45 sec at 72°C) and final extension for 5 min at 72 °C.

PCR products were visualized using gel electrophoresis. Agarose gel was prepared using 2% agarose made in 1x TAE buffer containing red gel (2.5 µl). After loading the amplicons in each well, an electric current of 150 MA and 100 V was applied for 40 min. Positive results were indicated by the presence of a 496 base pair band seen on the gel with an ultraviolet illuminator and taking the gel image using Bench top 2UV trans-illuminator. One confirmed *Salmonella* isolate from each positive sample was stored at -80°C in 20% glycerol until further testing

3.4. Antimicrobial sensitivity tests

Antimicrobial susceptibility of *Salmonella* isolates was conducted using standard Kirby-Bauer disk diffusion method according to clinical Laboratory Standards Institute (CLSI, 2013) guideline. Bacterial inoculums were prepared by suspending the fresh grown bacteria in sterile nutrient broth and the turbidity was adjusted to a 0.5 McFarland standard and carefully spread over the Muller- Hinton Agar plate using sterile cotton swab. Then panels of antimicrobials namely amikacin(AN), cefoxitin (FOX),

ceftriaxone (CRO), cephalothin (CF), chloroamphenicol (C), ciprofloxacin (CIP), gentamycin (GM), kanamycin(K), nalidixic acid (NA), neomycine (N), nitrofurantoin (F/M), streptomycin (S), sulfisoxazole (G), sulphamethoxazole +trimethoprim (SMT), tetracycline (TE), trimethoprim (TRM) and amoxycillin (AML) were placed on the agar plate. The plates were incubated aerobically at 37°C for 18–24 hours and zone of inhibition around each disc was measured using transparent ruler and used to classify the organisms as sensitive, intermediate or resistant according to cut off point set by CLSI (CLSI, 2013).

3.5. Data Management and Analysis

The collected data were entered and managed in MS Excel (Microsoft excel 2010) and Stata 12 software (Stat Corporation, 2012) was used for the data analysis.

Descriptive statistics were used to understand the nature and the characteristics of the data to show the rate of *Salmonella* detection in each sample type and distribution of *Salmonella* isolates. Chi square (χ^2), t-test and one way ANOVA were employed to assess presence of statistically significant difference between variables (species, sex, market areas, body condition and breeds). P-value <0.05 was considered significant.

4. RESULTS

4.1. Occurrence of *Salmonella*

A total of 234 sheep and 60 goats for fecal samples, 117 carcass swab samples (n= 85 for sheep and n=32 for goats), 10 meat samples (n= 6 for sheep and n=4 for goats) and 9 feed samples were cultured for *Salmonella*.

As shown in Table 3, the overall occurrence of *Salmonella* in sheep and goat fecal samples at Addis Ababa livestock market was 4.08% (12/294). Its occurrence in carcass swab samples collected from Addis Ababa public abattoir was 0.85% (1/117). Though, relatively higher occurrence of *Salmonella* was found in sheep (4.7%) than goats' (1.66%) fecal samples, there was no statistically significant difference ($p>0.05$) between these two species. And also there was no significant difference ($p>0.05$) in occurrence of *Salmonella* between the carcass swab samples of goats (3.12%) and sheep (0.0%). *Salmonella* was not isolated from any of the sheep carcass swab samples (n=85) collected from Addis Ababa public abattoir.

Table 3. *Salmonella* occurrence with respect to species, sex and body condition of sheep and goats

Sample type and other factors	No of sample cultured	No of positive (%)	Chi²	p-value
Fecal Sample				
Sheep	234	11 (4.70%)	1.123	0.289
Goat	60	1(1.67%)		
Total	294	12 (4.08%)		
Carcass Swab Sample				
Sheep	85	0(0%)	2.6791	0.102
Goat	32	1(3.1%)		
Total	117	1(0.85%)		
Sex factor				
Male	236	11(4.7%)	1.0257	0.311
Female	58	1(1.72%)		
Total	294	12 (4.08%)		
Body condition factor				
Good	178	10(5.6%)	2.7199	0.102
Poor	116	2(1.72%)		
Total	294	12(4.08%)		

Occurrence of *Salmonella* in fecal samples collected at four Addis Ababa livestock markets and Addis Ababa abattoir during anti mortem examination was 4.66% in male and 1.72% in females. But no statistically significant difference ($p>0.05$) was observed between the sexes. Similarly, the occurrence of *Salmonella* in fecal samples collected at four Addis Ababa livestock markets and Addis Ababa abattoir during anti mortem examination in animals with poor body conditioned animals,(1.72%) and good body conditioned animals ,(5.6%) or was not significantly different ($p>0.05$). The body condition was categorized as poor (score 1 and 2) and good (score 3 and above) was based on the guideline presented in Table 1 in materials and methods section.

4.2. Isolation of *Salmonella* from different sources

Salmonella was identified from 13(3.023%) samples of which 92.3% were from fecal and 7.7% were from carcass swab samples. *Salmonellae* was isolated from 12 (4.08%) of 294 fecal samples and only one isolate was obtained from 1 (0.85%) of 117 carcass swab samples; but no *Salmonella* was detected from the 10 meat and 9 feed samples. The proportion of positive samples therefore ranged from 0% in meat and feed to 4.08% in fecal (Figure 1).

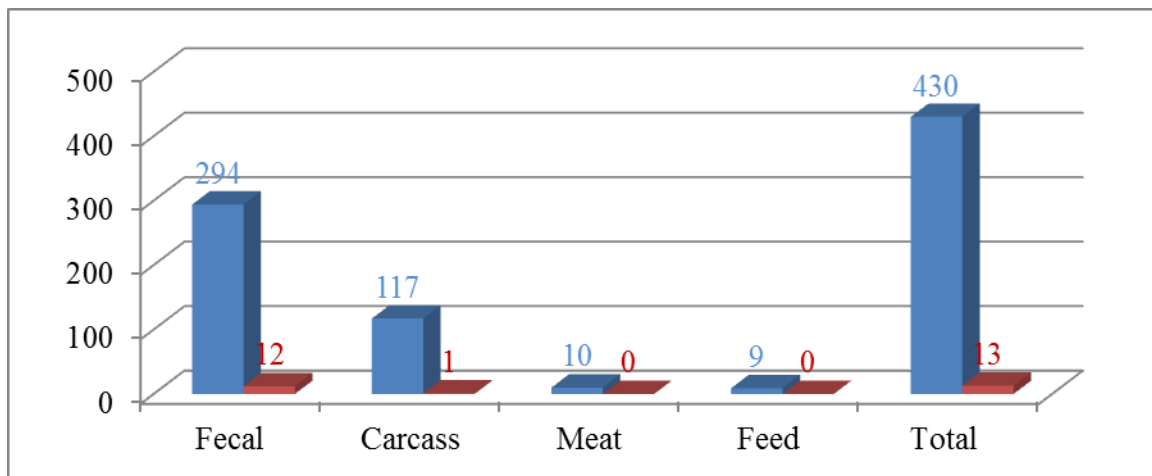


Figure 1. Sample types with their respective observations versus occurrence of *Salmonella*

4.3. Polymerase Chain Reaction (PCR) result

A total of 12 *Salmonella* isolates were confirmed by biochemical tests and PCR. Fig. 2 shows the PCR Gel image of the twelve *Salmonella* isolates confirmed by PCR out of the 20 tested.

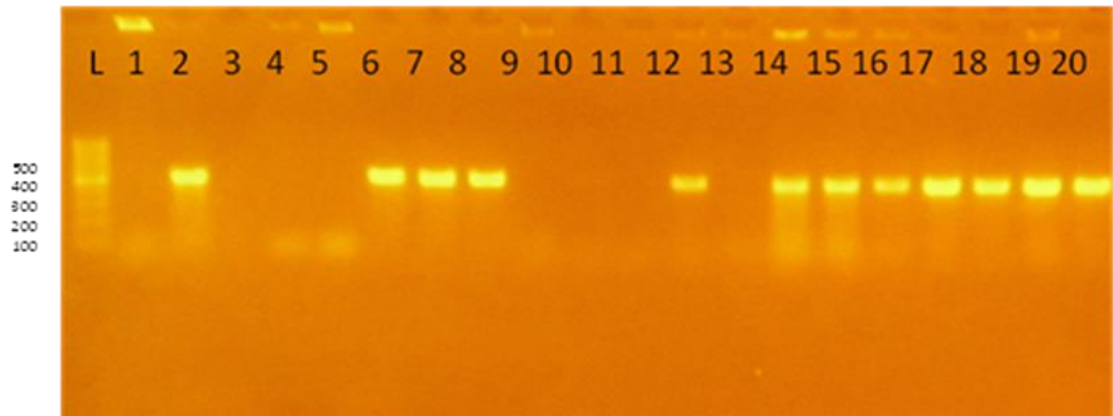


Figure 2. PCR amplification of suspected *Salmonella* isolates using genus specific PCR

Lane 1: 1kb ladder; Lane 1-20:samples; Lane 2, 6,7, 8, 12 and14-20 were positive for Genus *Salmonella* showing 496 bp amplification of target region of histidine transport operon; while Lane 1, 3, 4, 5, 9, 10, 11 and 13 were negative

4.4. Antimicrobial susceptibility of *Salmonella* isolates

A total of 13 *Salmonella* isolates recovered from sheep (n=11) and goats (n=2) were tested for antimicrobial susceptibility using 17 different antimicrobials (Table 5). Out of the thirteen isolates, 11 (84.6%) of the *Salmonella* isolates were resistant to streptomycin followed by gentamycin 6(67%), tetracycline 7(54%) and trimethoprim 7(53.8%). All the thirteen isolated *Salmonella* were susceptible to the four drugs (amikacin, cefoxitin, chloroamphenicol and nalidixic acid). Good susceptibility to 92.3% of the isolates was also recorded for each of ceftriaxone and nitrofurantoin. Intermediate resistance was

recorded towards cephalothin, neomycin and ciprofloxacin in 23.08%, 15.38% and 15.38% of the isolates respectively.

Table 4. Antimicrobial susceptibility profile of *Salmonella* isolated from sheep and goat fecal and carcass swab samples.

S.No.	Antimicrobials tested	Status of antimicrobial agent against the isolates		
		Resistant (%)	Intermediate (%)	Susceptible (%)
1.	Amikacin(AN)	0(0.0)	0(0.0)	13(100)
2.	Cefoxitin (FOX)	0(0.0)	0(0.0)	13(100)
3.	Ceftriaxone (CRO)	0(0.0)	1(7.69)	12(92.3)
4.	Cephalothin (CF)	1(7.69)	3(23.07)	9(69.23)
5.	Chloroamphenicol (C)	0(0.0)	0(0.00.0)	13(100)
6.	Ciprofloxacin (CIP)	0(0.0)	2(15.3)	11(84.61)
7.	Gentamycin (GM)	6(66.7)	0(0.0)	3(33.3)
8.	Kanamycin(K)	5(38.5)	0(0.0)	8(61.53)
9.	Nalidixic Acid (NA)	0(0.0)	0(0.0)	13(100)
10.	Neomycin (N)	2(15.4)	2(15.3)	9(69.23)
11.	Nitrofurantoin (F/M)	1(7.69)	0(0.0)	12(92.3)
12.	Streptomycin (S)	11(84.62)	1(7.69)	1(7.69)
13.	Sulfisoxazole (G)	5(38.46)	0(0.0)	8(61.53)
14.	Sulphamethoxazole and Trimethopirim (SMT)	3(42.86)	0(0.0)	4(57.14)
15.	Tetracycline (TE)	7(53.84)	0(0.0)	6(46.15)
16.	Trimethoprim (TRM)	7(53.84)	0(0.0)	6(46.15)
17.	Amoxycillin (AML)	3(23.07)	0(0.0)	10(76.92)

53.85% of the *Salmonella* isolates from sheep and goats were multiple resistant to four to nine antimicrobial drugs, *Salmonella* isolates showed substantial resistance to

streptomycin(84.62%), ampicillin(77%), Gentamycin(67%), tetracycline(54%) and trimethoprim(53%)(Table 6).

Of the 13 *Salmonella* isolates examined, 8 (61.5%) of them were resistant to 3 or more antimicrobials, 4(30.6%) of them were resistant to 6 or more number of antimicrobials whereas other 4 (30.6%) of the isolates were resistant to two antimicrobials. All of the isolates tested were resistant to at least one antimicrobial agent examined. Antimicrobial resistance profile of *Salmonella* isolates examined in the current study is shown in Table 6.

Table 5. Antimicrobial resistance pattern of the *Salmonella* isolates

Isolate ID	Resistance Pattern	No. of Drug to which isolate is resistant	No of isolates
417	CF,K,S,G,SMT,TE,TRM and AML	8	2
447	GM,K,N,S,G,SMT,TE and TRM	8	
445	K,N,S,G,TE,TRM	6	2
31	K, S, G,TE,TRM and AML	6	
317	S, G, TE, TRM and AML	5	1
439	GM, S,TE and TRM	4	1
2	GM,S and TRM,	3	2
400	K and S	2	4
78	GM, and TE	2	
303(A)	F/M and S	2	
10	GM and S	2	
12	GM	1	2
19	S	1	

CF-cephalothin, K-kanamycin, S-streptomycin, G- sulfisoxazole, SMT- sulphamethoxazole and trimethopirim, TE- tetracycline , TRM- Trimethoprim, AML- amoxycillin, GM- gentamycin , N- neomycin, F/M- nitrofurantoin

5. DISCUSSION

Higher isolation of *Salmonella* was found in sheep (4.7%) than goats (1.66%) fecal samples, this higher occurrence in sheep might be due to differences in feeding behavior between sheep and goat, sheep prefer to graze while goat to browse and rearing area as well as management differences. Close contact and holding time during journey and at arrival in market area which predispose to cross contamination through poor hygiene of the market environment. Though feed samples were negative in this study which might be due to small sample size; despite the fact that, it needs further investigation to identify clearly the factors which are responsible for the variation.

Though relatively high *Salmonella* occurrence was observed in male and good body conditioned animals compared to females and animals with poor body condition, there was no significant difference. The observed minor difference could be attributed to the difference in sample size in both cases. The probable reason why *Salmonella* prevalence was low in carcass swab samples compared to fecal samples of small ruminants in the current study could be due to good hygienic practices if the abattoir workers with minimal contamination of the carcass with fecal material.

Salmonella isolation rate of 4.7% in fecal samples of sheep in this study was in agreement with different findings: 4.17% in addis ababa livestock market and abattoir enterprise by Tigist (2017), 3.3% from sheep feces in Jimma, (Anbessa and Katema, 2012) 4.8% isolates in the fecal samples of apparently healthy slaughtered sheep in Debrezeit abattoirs and sheep and goat in central Ethiopia(Molla *et al.*, 2006) and 4.8% in Ethiopia by Wassie (2004), However, it was relatively higher than the 1.04% isolation rate by Bedaso *et al.* (2015) who reported from different organs and feces of apparently healthy sheep and goats slaughtered at Addis Ababa Abattoir Enterprise and 2.1% in Ethiopia (Woldemariam, 2003). The 1.7% prevalence in goat feces in this study was lower than that of 3.3% in Ethiopia (Woldemariam, 2003). The occurrence of *Salmonella* (0.85%) in carcass sample in this study was lower than the findings of Woldemariam

(2003) accounting for 6.4% at Elfora Debre Zeit abattoir in Ethiopia and Wassie (2004) which also reported 11.5% for sheep and 3% for goats. 6.19% Isolation of Non-Typhoidal *Salmonella* from Sheep feces in Eastern Hararghe, (Zweifel *et al.*, 2004), 7.7% from the fecal samples of sheep slaughtered (Teklu and Negussie, 2011) in Export Abattoir of Modjo Ethiopia. The difference in the prevalence could be due to differences in study period, study site (abattoirs), study population and number of samples examined between the studies.

In the present study 53.85% of the *Salmonella* isolates from sheep and goats were multiple drug resistant to antimicrobial drugs commonly used to treat bacterial infections in domestic animals and humans in Ethiopia. This finding supports the well known fact that antimicrobial resistance rate of *Salmonella* isolates in Sub-Saharan Africa is generally high which is primarily attributed to the indiscriminate use of antimicrobials both in animals and human health sectors (Leegaard *et al.*, 1996; Molla *et al.*, 1999b).

Salmonella isolates showed substantial resistance to streptomycin, ampicillin, gentamycin, tetracycline and trimethoprim. It has been suggested that emergence of multiply-resistant salmonellae can be the product of conjugative transfer of R-plasmids between bacterial species. Agricultural use of sub therapeutic doses of a single antibiotic could select for bacterial strains harboring plasmids with multiple resistance codons (D'Aoust, 1991; D'Aoust, 1997). The emergence and prevalence of multiply-resistant salmonellae in meat animals can seriously compromise public health (D'Aoust *et al.*, 1992). This might limit therapeutic choice to manage salmonellosis and other bacterial diseases. In general, antimicrobial use is a key driver of resistance development, which could be irrational use of antimicrobials due to lack of access to appropriate treatment and underuse due to inadequate dosing, poor adherence or use of substandard antimicrobial and lack of financial support to complete treatment course.

6. CONCLUSION AND RECOMMENDATIONS

In this study the occurrence of *Salmonella* in sheep and goats was 4.08% and 0.85% respectively for the samples collected from Addis Ababa livestock markets and Addis Ababa public abattoir as well as butcher's shop of different sub-cities. Higher isolation was observed in feces of sheep and goats samples as compared to other sample types. *Salmonella* was not detected from feed and meat samples from butcher shops. To control and prevent *Salmonella* infection and contamination in live animals and animal products, it is critical that risk reduction strategies should be used throughout the food chain. Based on the findings of the present study, the followings are recommended:

- ✚ Even though the rate of occurrence of *Salmonella* in carcass is not that much higher, ligation of the esophagus and gut during evisceration and immediate separation of the offal from the carcasses should be employed, in order to reduce contamination from gastrointestinal content.
- ✚ Further investigation on characterization of the *Salmonella* isolates to identify the species and serotype should be carried out
- ✚ Further detailed studies should be conducted to undertake molecular characterization of resistant genes associated with the observed resistance phenotype
- ✚ Since antimicrobial resistance problem is expanding, appropriate national regulations and control strategies on use and application antimicrobial agents should be designed.

7. REFERENCES

- Abdel-Ghani, M., Mohomed, A. H., Yassein, S. (1987): Occurrence of *Salmonella* in sheep and goats in Egypt. *J. Egypt. Vet. Med. Assoc.* **47**, 161-170.
- Alemayehu, D. Molla, B. and Muckle, A. (2003): Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Hlth. Prod.***35**, 309-319.
- Anbessa, D. and Ketema, B. (2012): The prevalence and Antibigram of *Salmonella* and *Shigella* solated from abbatoir, Jimma town, south west Ethiopia. *Int. J. Pharm .Biol .Res*, 13-16.
- Anderson, A. (1996): Outbreak of *Salmonella* food poisoning at Junior World Rowing Championships. Dec; 30(4): 347–348.
- Anderson, E. and Williams R. E. O. (1956): Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J. Clin. Pathol.***9**, 94-114.
- Ashenafi, M. and Gedebeu, M. (1985): *Salmonella* and *Shigella* in adult diarrhea in Addis Ababa: prevalence and antibiograms. *Trans. Royal Society Trop. Med.Hyg.***79**, 719721.
- Baird-parker, A. (1990): Foodborn salmonellosis. *The Lancet*,**336**, 1231-1235.
- Barbara, M. Baird-Parker, T. and Grahame, W. (2000): The microbiological safety and quality of food (II). Gaithersburg, Maryland, USA: Aspen Publishers Inc. p.1234.
- Bedaso, K. Dinsefa, JH. Kifle, AL. and Biniam, T. (2015): Study on prevalence and distribution of *Salmonella* isolates from apparently healthy sheep and goats slaughtered at addisababa abattoir enterprise, Ethiopia. *J. Veterinary. Sci .Technol* ,**6**: 268.

- Blackburn, C. (1993): Rapid and alternative methods for the detection of salmonellas in foods. *J. Appl. Bacteriol.* **75**, 199-214.
- Brenner, F. McWhorter-Murlin, A. (1998): Identification and serotyping of Salmonella. Centers for Disease Control and Prevention, Atlanta.
- Brenner, F.W. Villar, R.G. Angulo, F.J. Tauxe, R. Swaminathan, B. (2000): Salmonella nomenclature. *J Clin Microbiol* 38, 2465-2467.
- CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) (2013): An Atlas of Salmonella in the United States, 1968–2011: Laboratory-based Enteric Disease Surveillance. Atlanta, Georgia: US Department of Health and Human Services, CDC, Atlanta, Georgia, USA.
- Choudhary, A. Gopalakrishnan, R. Senthur, P. Ramasubramanian, V. AbdulGhafur, K. and Thirunarayan, M. (2013): Antimicrobial susceptibility of *Salmonella enterica* serovars in a tertiary care hospital in southern India.
- Clark, P. D. and Pazdernik, J. N. (2013): Polymerase Chain Reaction, Molecular Biology 2nd edition, Elsevier Inc. Pp. 55 -61.
- Clarke, R. C. and Gyles, C. L. (1993): *Salmonella*. In: Gyles, C. L. and Thoen, C. O. (eds.): Pathogenesis of Bacterial Infections in Animals. 2nd ed. USA: Iowa State University press, Ames. PP. 133-153.
- CLSI, (2013): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement M100-S23.
- Cohen, ND. Neibergs, HL. McGruder, ED. Whitford, HW. Behle, RW. Ray, PM. Hargis, BM. (1993): Genus-specific detection of salmonellae using the polymerase chain reaction (PCR). *J Vet Diagn Invest*, 5(3):368-371.
- Cortesi, M. L. Brillante, M. Giovanni, F. DE. (1984): *Salmonella* carriage by apparently healthy slaughter sheep and goats. *Archivio Vetrinario Italiano*. **35**, 258-261.

- Craig, D. and James, M. (2006): The genus *Salmonella* In: The Prokaryotes 3rd Edition. **6**: 123- 158.
- D'Aoust, J.-Y. (1991): Pathogenicity of foodborne *Salmonella*. *Int. J. Food Microbiol.* **12**, 17- 40.
- D'Aoust, J.-Y. (1997): *Salmonella* Species In: Doyle, M. P., Beuchat, L. R., Montville, T. J. (eds.): Food Microbiology Fundamentals and Frontiers. Washington: Asm Press. PP. 129-158.
- D'Aoust, J.-Y. Sewell, A. M. Dally, E. and Greco, P. (1992): Antibiotic resistance of agricultural and foodborne *Salmonella* isolates in Canada: 1986-1989. *J. Food Prot.* **55**, 428-434.
- Doyle, M. and Cliver, D. (1990): *Salmonella* In: Cliver, D. O. (ed): Foodborne diseases. California: Academic Press, Inc. PP. 185-204.
- Dresse, H. Aschalew, G. Wassie, M. Legesse, G. Linda, C. (2015): Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia
- Ejeta, G. Molla, B. Alemayehu, D. and Muckle, A. (2004): *Salmonella* serotypes isolated from minced beef, mutton and pork in Addis Ababa, Ethiopia. *Rev. Med. Vet.*
- Enyew, M. Hiskel, B. and Belay, W. (2016): Isolation, Identification and Drug Resistance Profile of salmonella from Apparently Healthy Cattle Slaughtered at Wolaita Sodo Municipality Abattoir, Ethiopia. *Journal of Biology, Agriculture and Healthcare*. Vol.6, No.13, 2016
- Ewing, W. H. (1986): Serological Identification of *Salmonella*. In: Ewing, W. H. (ed): Edwards and Ewing's Identification of *Enterobacteriaceae*. 4thed. New York: Elsevier science publishing Co., Inc. PP. 201-238.
- Fluckey, W. Loneragan, G. Warner, R. and Brashears, M. (2006): Antimicrobial Drug Resistance of *Salmonella* and *Escherichia coli* Isolates from Cattle Feces, Hides, and Carcasses. *Journal of Food Protection*, Vol. 70, No. 3, 2007, Pages 551–556

- Fufa, A., Ashenafi, F., Haile, J., Takele, B., Reta, D.(2017): Isolation, identification and antimicrobial susceptibility profile of salmonella isolates from abattoir and dairy farms in and around Holeta town, Oromia, *College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia*.
- Girma, A. Yami, A. and Merkel, C. (2007) *Body Condition Scoring Of Sheep And Goats, Technical Bulletin No.8, Ethiopia Sheep and Goat Productivity Improvement Program*
- Gizaw, S. Yami, A. Awgichew, K. Gipson, A. and Merkel, C. (2009): *Sheep breeds of Ethiopia: A guide for identification and utilization, Technical Bulletin No.28. Ethiopia Sheep and Goat Productivity Improvement Program*
- Hailu, T. and Kebede, B. (2016): Isolation of Non-Typhoidal *Salmonella* from Sheep faeces in Eastern Hararghe, Ethiopia Appli Micro Open Access, 2:3
- Hans, P. Dean, O. and Morris, P. (2006): *Salmonella* infection In: Foodborne Infections and Intoxications 3rd Edition. Food Science and Technology International Series: 57-136.
- Hendriksen, R. (2002): A global Salmonella surveillance and laboratory support project of the world Health Organization: Laboratory protocols (susceptibility testing of *salmonella* using disk diffusion), 3rd edition.
- International Organization for Standardization (ISO) (1998): Microbiology of Food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonella*. ISO. 6579. Geneva.
- International Organization for Standardization (ISO) (1998): Microbiology of Food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonella*. ISO. 6579. Geneva.
- Jawetz, E. Adelberg's, E. A. Mlnick, J. L. (1995): Medical Microbiology. 20thed. Appleton and Large. PP. 206-217.

- Jay, J. (2000): Modern Food Microbiology. 6thed. Maryland: Aspen publication. PP. 511-525.
- John, S. (2011): Department of Agriculture and Food Body condition scoring of sheep and goat. Sheep Industries Branch, South Perth, Melbourne.
- Judicial Commission of the International Committee on Systematic of Prokaryotes. Microbiol 2005; 55:519-520.
- Kapur, M. P., Kulshreshtha, R. C., Kalra, D. S. (1982): *Salmonella* serotypes from sheep and their significance. *Indian Vet. J.* **59**, 331-334.
- Kaura, Y. K. and Sharma, V. K. (1978): Isolation of salmonellae from lambs. *Indian J. Experimental Biology*, **17**, 448-450.
- Kemal, J. (2014): A Review on the Public Health Importance of Bovine Salmonellosis. *J VeterinarSciTechnol* 5: 175.
- Khakhria, R., Woodward, D. Johnson, W. Poppe, C. (1997): *Salmonella* isolated from humans, animals and other sources in Canada during the period 1983-1992. *Epidemiol. Infect.* **119**, 15-23.
- Kimberling, C. (1988): Jensen and Swift's Diseases of Sheep. 3rd ed. Philadelphia: Lea and Febiger. PP. 54-55, 153-154.
- Kotova, A. L., Kondratskaya, S. A., Yasutis, I. M. (1988): *Salmonella* carrier state and biological characteristics of the infectious agent. *J. Hyg., Epid., Micro. Immu.* **32**, 71-78.
- Krauss, H. Albert, W. Appel, M. Henery, B. Lesenberg, D. (2003): Zoonosis infectious disease from animals to humans. 3rd edition. American Society, for Microbiology: 234-236.
- Kumar, S. (2012): Text Book of Microbiology, 1st Edition. J.B Medical Publishers(p) Ltd, New Delhi, India.

- Kumar, S. Saxena, S. and Gupta, B. (1973): Carrier rate of salmonellas in sheep and goats and its public health significance. *J. Hyg. Comb.* **71**, 43-47.
- Leegaard, T. M. Van Gestel, M. H. Petit, P. L. C. Van De Klundert, J. A. M. (1996): Antibiotic resistance mechanisms in *Salmonella* species causing bacteraemia in Malawi and Kenya. *APMIS*, **104**, 104-306.
- Mache, A. (2002): *Salmonella* serogroups and their antibiotics resistance patterns isolated from diarrheal stools of pediatric outpatient in Jimma Hospital and Jimma Health Center, South West Ethiopia. *Ethiop. J. Health Sci.* **12**, 37-46.
- Madhuri, D. Gupta, V. Nema, S. Patidar, A. Shivhare, M. Singh, N. and Shakya, V. (2012): Modern semen evaluation techniques in domestic animals: A review. DHR International *Journal of Biomedical and Life Sciences* (DHR-IJBLS) ISSN: 2278-8301, Vol. 3(1).
- Mastroeni, P., Maskell, D., 2006, *Salmonella* infections : clinical, immunological, and molecular aspects. Cambridge University Press, Cambridge.
- Molla, B. Alemayehu, D. and Salah, W. (2003): Source and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. *Ethiop. J. Health Dev.* **17**, 63-70.
- Molla, B. Kleer, J. and Sinell, H. (1999): Occurrence, distribution and level of *Salmonella* in selected food items in Addis Ababa (Ethiopia). *Fleischwirtschaft. International*, 37-39.
- Molomo, M. (1998): A study on the prevalence and epidemiological key factors of *Salmonella* species in selected poultry farms in DebreZeit, Ethiopia. Freie Universität Berlin and Addis Ababa University, MSc. Thesis.
- Nabbut, N. and Al-Nakhli, H. (1982): Incidence of salmonellae in lymph nodes, spleen and faeces of sheep and goats slaughtered in the Riyadh public abattoir. *J. Food Prot.* **45**, 1314-1317.
- Nyeleti, C. Hildebrandt, G. Kleer, J. and Molla, B. (2000): Prevalence of *Salmonella* in Ethiopian cattle and minced beef. *Berl. Munch. Tierärztl. Wschr.* **113**, 431-434.

- Office International des Épidémiologies (OIE) (2000): Salmonellosis. In: Manual of Standards for Diagnostic Tests and Vaccines, OIE. Paris. PP. 832-842.
- Office International des Épidémiologies (OIE) (2000): Salmonellosis. In: Manual of Standards for Diagnostic Tests and Vaccines, OIE. Paris. PP. 832-842.
- Office International des Épidémiologies (OIE) Terrestrial Manual (2016): Salmonellosis. Version 1 adopted by the World Assembly of Delegates of the OIE in May 2016
- Orellana, B. (2012): Longitudinal Study Of *Salmonella Enterica*, *Escherichia Coli* O157:H7, and *Listeria Monocytogenes* in a Small and Very Small Fresh Meat Processing Plant Environment, Colorado State University Fort Collins, Colorado.
- Pegram, R. Roeder, P. Hall, M. and Rowe, B. (1981): *Salmonella* in livestock and animal byproducts in Ethiopia. *Trop. Anim. Hlth. Prod.* **13**, 203-207.
- Plym, F. and Wierup, M. (2006): Salmonella contamination: a significant challenge to the global marketing of animal food products. *Rev. Sci. tech. Off. Int. Epiz.* **25**: 541-554.
- Popoff, MY. and Le Minor, L. (1997): Antigenic Formulas of the *Salmonella* Serovars. 7th ed. WHO Collaborating Centre for Research on *Salmonella*. Institute Pasteur. Paris, France.
- Popoff, MY. Bockemühl, J. Gheesling, L. (2004): Supplement 2002 (no. 46) to the Kauffmann-White scheme. *Res Microbiol*; **155**:568-570.
- Popoff, MY. Le Minor L. (2001): Antigenic formulas of the *Salmonella* serovars, 8th revision, World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, Pasteur Institute, Paris, France..
- Publications Board. Publications Board meeting minutes (1999): *Salmonella* nomenclature. *ASM News*; **65**:769.
- Quinn, P. Carter, M. Markey, B. and Carter, C. (1994): *Veterinary Clinical Microbiology*. London: Wolfe Publishing. PP. 226-234.

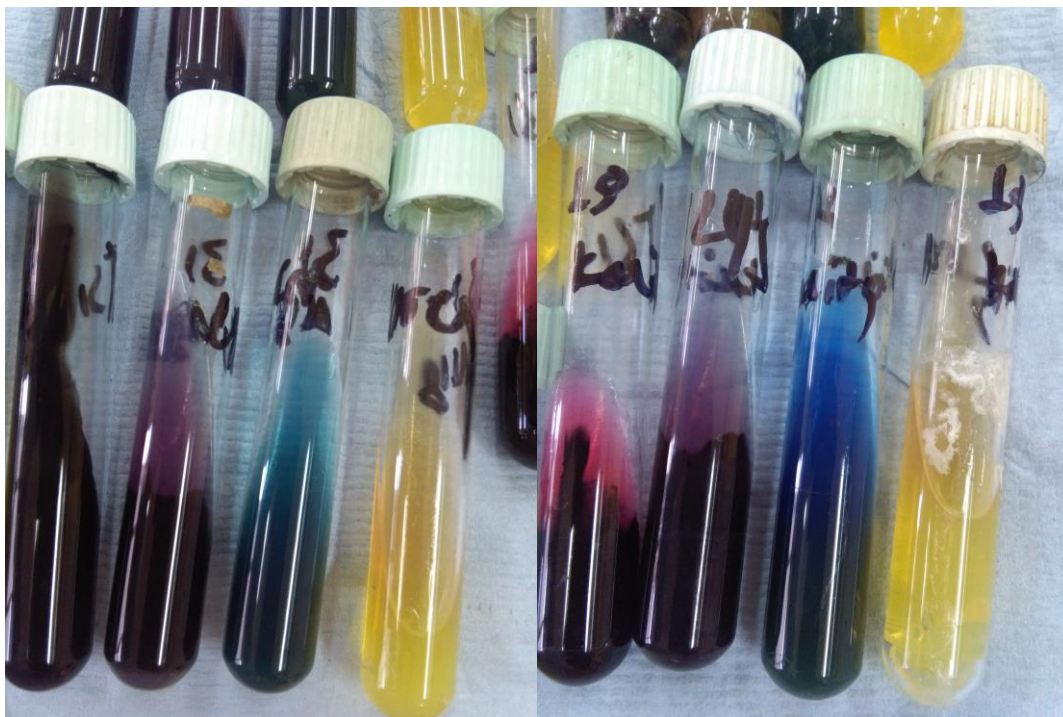
- Radostitis, O. Gay, C. Hinchliff, K. and Constable, P. (2007): Veterinary Medicine: A text book of the disease of cattle, horses, sheep, pigs, and goats. 10th ed. Elsevier Ltd. 325-326.
- Radostits, O. Blood, D. and Gay, C. (1994): Veterinary Medicine: A Text Book of the Disease of Cattle, Sheep, Pigs, Goats and Horse. 8th ed. London: BailliereTindall. PP. 730-747.
- Rao, P. (2004): Essential of Microbiology. Satish Kumar Jain for CBS publishers and Distributors, New Delhi. India. 146-148.
- Seifert, H. (1996): Tropical Animal Health. Dorotrecht: Kluwer Academic Publishers. PP. 368-371.
- Shelobolina, E. Sullivan, S. O'Neill, K. Nevin, K. Lovley, D.(2004): Isolation, characterization, and U(VI) reducing potential of a facultatively anaerobic, acid-resistant bacterium from low-pH, nitrate- and U(VI) contaminated subsurface sediment and description of *Salmonella subterranea* sp. nov. *Appl Environ Microbiol*; 70:2959-2965.
- Smith, M. and Sherman, D. (1994): Goat Medicine. Maryland: Williams and Wilkins. PP. 302-305.
- Stata Corporation (2012): Stata Stastical Software. Release 12. College Station, Texas.
- Tegegne, M. and Ashenafi, M. (1998): Microbial load and incidence of *Salmonella* Spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish. *Ethiop. J. Health Dev.* **12**, 135-140.
- Teklu, A. Negussie, H. (2011): Assessment of risk factors and prevalence of *Salmonella* in slaughtered small ruminants and enviroment in an export abattoir, Modjo, Ethiopia. *American-Eurasian J. Agric. Environ. Sci*, **10**(6):992-999.
- Terzolo, H. R., D'Empaire, M. Bendersky, S. Igarza, L. M. (1979): Prevalence of *Salmonella* Typhimurium in clinically healthy flock of sheep. *Revista de Medicina Veterinaria, Argentina*, **60**, 277-282.

- Threlfall, E. Ward, L. Frost, J. and Willshaw G., (2000): The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology*. 62:1-5.
- Thrusfield, M. (1995). *Veterinary Epidemiology*. 2nded. Oxford: Black Well Science Ltd. PP.182-198.
- Tibaijuka, B. Molla, B. Hildebrandt, G. and Kleer, J. (2003): Occurrence of salmonellae in retail raw chicken products in Ethiopia. *Berl. Munch. Tierärztl. Wschr.* **116**,55-58.
- Tigist, SH., Abebe, A., Taddese, E., Haile, A.(2017): Prevalence and antimicrobial susceptibility of *salmonella* in apparently healthy sheep and goats brought at addis ababa livestock market and abattoir enterprise. *Jigjiga University, College of Veterinary Medicine*, Ethiopia.
- Varnam, A. and Evans, M. (1991): *Foodborne Pathogens: An Illustrated Text*. England: Wolfe Publishers Ltd. PP. 51-85.
- Ward, L. DeSa, J. and Rowe, B. (1987): A phage-typing scheme for *Salmonella* Enteritidis. *Epidemiol. Infect.* **99**, 291-294.
- Wassie, m. (2004): A cross-sectional study on salmonella in apparently healthy slaughtered sheep and goats at addis ababa and modjo abattoirs, Ethiopia. A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University for partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine
- Woldemariam, E. (2003): Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in DebreZeit, Ethiopia. AAU, FVM, DebreZeit, Ethiopia, DVM. Thesis.
- World Health Organization (1988): *Salmonellosis Control: Role of animal and product hygiene*. Report of a WHO Expert Committee. Geneva: WHO Technical Report Series, No. 774.

- Wray, C and Linklater, K. (2000): *Salmonella* Infections in Sheep Veterinary Laboratories Agency (Weybridge), New Haw, Addlestone, *Salmonella* in Domestic Animals. CABI Publishing. UK.pp 209-214
- Wray, C. (1994): Mamalian salmonellosis In: Hand book of zoonosis 2nd Edition. Edited by Beran GW, New York, C.R.C, press: 291-300.
- Wray, C. and Davis, R. (2000): *Salmonella* infection in cattle. In: Wray, C. and Wray, A. (eds.): *Salmonella* in Domestic Animals. CABI Publishing UK. PP. 169-190.
- Wray, C. and Davis, R. (2003): The epidemiology and ecology of *Salmonella* in meat-producing animals. In: Torrence, M. E. and Isaacson, R. E. (eds): Microbiological Food Safety in Agriculture Current Topics. Iowa State: Black Well Publishing Company. PP. 73-82.
- Yan, S.Pandrak, M.Abela-Rider, B.Punderson, J. Fedorko, D. and Foley, S.(2003): An overview of *Salmonella* typing public health perspectives. *Clinical and Applied Immunology Reviews*. 4: 189-204.
- Zelalem, A.Nigatu, K. Zufan, W. Haile, G.Alehegne, Y. and Tesfu, K. (2011): Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: across sectional study. *BMC Inf. Dis.*, 11: 222.
- Zweifel, C., zycowska,MA. and Stephan, R. (2004): Prevalence and characteristics of shiga toxin producing *Escherichia Coli*, *Salmonella* spp. And *Campylobacter* spp. Isolated from slaughtered sheep in Switzerland. *Int. J. Food. Micro*, **92**: 45-53.

8. APPENDICES

Appendix 1: Salmonella colony morphology and its biochemical characteristics



Appendix 2: Questionnaire for investigation of fecal carriage of Salmonella in small ruminants marketed in Addis Ababa and their carcass

Owner name_____region_____
zone_____Woreda_____, How long have you been involved in this work
(small ruminant trade)_____Specific name of the market
place_____kebele_____Date_____

1. What do you feed your animals at market place? A. Hay, B. Pasture, c. Concentrate, other?
2. From where did you bring the animals?_____
3. At this market place, do you mix your animals with those from other places?_____
4. What was the means of transportation? A. vehicle; B. On foot. C, other
5. What is the maximum number of days your animals spend in this market place?_____
6. How many animals are there in the group when sample collection?_____
7. What was the total number of animals in your group when originally brought?_____
8. What type of disease is the major problem in your flock?_____
9. When is it more common? (season of occurrence)_____pathognomnic sign_____What do you do when your shoats got sick?
A. Get professional assistance; B. treat by myself
10. Is there any sick animal in your current group?_____If yes what is the sign? _____Have you treated them?
A.Yes ,B. no ; If yes what was the drug?_____
11. Have you ever encountered any death in your flock?_____
12. How you dispose the dead ones? A. Buried, B. burning, C. disinfecting the environment, D. leave for carnivores, E. others
13. How do you rate the hygiene of the environment? A. Poor B. Fair C. Good

Give sequential numbers for the specific animals from the flock in that specific market

ID	Species	Age	Body condition	Breed type	No. days in this market	Remark