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A cross sectional study on *Salmonella* in apparently healthy sheep and goats slaughtered at Elfora and Luna export abattoirs, Ethiopia

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A cross-sectional study was conducted between November 2015 and March 2016 on apparently healthy slaughtered sheep and goats, and clean knife at Luna and Elfora export abattoirs located at Modjo and Bishoftu towns to estimate the prevalence of *Salmonella* in sheep and goats, to assess the hygienic condition of flaying knife, and to isolate and identify the prevalent *Salmonella* sub-species. A total of 525 samples consisting of cecum (n=122), liver (n=122), mesenteric lymph nodes (n=122), abdominal muscle (n=122) from 44 sheep and 78 goats and 37 pooled knife samples were collected. The samples were examined for the presence of *Salmonella* following the conventional techniques of ISO standard and using OMNILOG bacterial identification system, GEN III microplate for confirmation and sub species identification. From the total of 122 animals examined, 21 (17.21%) were positive of which 12 (9.83%) were sheep and 9 (7.38%) were goats, and none of the samples from pooled knife swabs were positive for *Salmonella*. Statistically significant difference (P=0.04) in the prevalence of *Salmonella* was observed between the two species. The frequency of isolation was 10 (3.89%) and 11 (4.10%) from Luna and Elfora abattoirs, respectively. As a result, there was no significant difference (P =0.884) in the prevalence of *Salmonella* isolation between the two abattoirs. Of the total 488 tissue samples examined from apparently healthy slaughtered sheep and goat, 21 (4.3%) samples were *Salmonella* positive. *Salmonella* was isolated from 6.56% mesenteric lymph nodes, 5.73% cecum, 4.09% liver and 0.82% abdominal muscle samples. However, there was no significant difference between tissues (P=0.13). From the 21 isolated *Salmonella* species, 20 of them were confirmed to be the pathogenic *Salmonella enterica* subspp. *enterica* and 1 isolate was the non-pathogenic *Salmonella enterica* subspp. *salamae*. The results of this study showed the potential risk of sheep and goats as sources of pathogen for humans in the study area. These findings stressed the need for implementation of preventing close contact of offal and carcass during evisceration.

Key words: Elfora, goats, knife, luna, prevalence, *Salmonella*, sheep, sub species.

INTRODUCTION

Sheep and goats in Africa are noted for their ability to convent low opportunity cost feed into high value products, namely, meat, milk, fiber, manure and skin (Wilsmore, 2006). In Ethiopia their population is

estimated to be about 28.89 million sheep and 29.70 million goats (CSA, 2016).

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause spoilage and food borne infections in human, resulting in economic and health losses (Komba et al., 2012). A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature and transportation means of raw meat (Li et al., 2006; Adu-Gyamfi et al., 2012). Specific sources that contribute microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include the faeces, the skin, water, air, intestinal contents, lymph nodes, processing equipment and humans, and can be transferred to the carcass during skin removal and evisceration (Hansson et al., 2000; Reid et al., 2002).

Active surveillance data on foodborne diseases from the United States revealed that among pathogens associated with foodborne outbreaks, *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter*, and *Listeria monocytogenes* are responsible for the majority of outbreaks (Chen and Jiang, 2014). *Salmonella* is among the major causes of meat contamination that can affect small ruminant as well as human being (Pepin et al., 1997; Sierra et al., 1995). The members of the genus *Salmonella* are Gram-negative, motile, facultative anaerobic, bacilli belonging to the family Enterobacteriaceae. They comprised two central species, *Salmonella enterica* and *Salmonella bongori*. Presently, six subdivisions of *S. enterica* subspecies I-VI exist with over 2500 serovars currently identified and several common serovars to human clinical infections (Dworkin et al., 2006).

Diagnosis of salmonellosis is based on the isolation of the organism either from tissues collected aseptically at the necropsy or from feacal, rectal swabs or environmental samples. It can be isolated by standard cultural techniques and various biochemical and serological tests (OIE, 2000).

Previous studies conducted in Ethiopia on salmonellosis indicated the existence of the infection in various animal species (poultry, cattle, camels, sheep and goats, fish), in retail food items (minced beef, chicken meat and offal) (Mache et al., 1997; Molla et al., 1999, 2003; Nyeleti et al., 2000; Woldemariam, 2003; Gebremedhin, 2004; Ferede et al., 2015). However, in most of the studies *Salmonella* species identification were carried out by using conventional bacteriological tests in which it is difficult to identify pathogenic from non pathogenic salmonella species, where both can show similar properties by the traditional tests. Moreover, the information on the prevalence of pathogenic species of salmonella from sheep and goat, from Elfora and Luna export abattoirs and the zoonotic importance of sheep and goat salmonellosis is not as much known. Therefore, the objectives of this study were to estimate the occurrence of *Salmonella* in different organs and flaying knife of sheep and goat and to identify the prevalent *Salmonella* subspp. Using OMNILOG bacterial identification system.

MATERIALS AND METHODS

Study area and population

The study was conduct at Elfora export abattoir in Bishoftu and Luna export abattoir at Modjo, Ethiopia. The study animals were apparently healthy sheep and goats slaughtered at Elfora and Luna export abattoirs and flying knifes used for slaughtering of sheep and goats by the two abattoirs that can possibly contamination the carcasses.

Study design, sampling methods and sample size determination

A cross-sectional study was carried from November 2015 to March 2016 to isolate *Salmonella* spp. from slaughtered sheep and goats. Individual animals were sampled by using systematic random sampling depending on the number of animals slaughtered on each day. Samples were collected with interval of two weeks and each visit 10 animals and three pooled samples of knife were sampled. From each selected slaughtered sheep and goats, cecum, carcass (abdominal muscle), liver, mesenteric lymph node, and swabs from knife were collected. Sample size was calculated by considering expected prevalence of 8.7% (Teklu and Negussie, 2011), 5% desired absolute precision and 95% confidence interval using the formula recommended by Thrusfield (2007). Accordingly, the minimum sample size was 122.

$$n = Z^2 P \exp (1\text{-}Pexp) / d^2$$

Where, n is required sample size; Pexp is the expected prevalence (8.7%), d is the desired absolute precision (5%), and Z = 1.96.

Study methodology

Sample collection

All samples were collected aseptically using sterile forceps and scalpel blades from sheep and goats during slaughtering operation. From each selected animal, sufficient amount of samples cecum, carcass (abdominal muscles), liver and mesenteric lymph node were collected separately in sterile universal bottles. As soon as the abdomen of the animal was opened, the intestine with the mesenteric lymph nodes were separated from the rest of gastrointestinal tract and kept in a separate clean container until the other tissue samples collection from the same animal have been completed. Samples from knife were collected aseptically using sterile cotton swabs and samples were taken as soon as they had slaughtered the first animal and passed to the second animal. In

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both abattoirs, the slaughter personnel did immerse their knife in hot water in every slaughtered cycle and the collected swab samples were immersed in peptone water for transportation to laboratory. Collected samples were labeled uniquely identifying name of abattoirs, species of the animal, type of sample, date of sampling and sample ID number. All the collected samples were transported by cold chain and delivered to the general microbiology laboratory of National Animal Health Diagnostic and Investigation Center/NAHDIC/ within 24 h for bacteriological processing.

Bacterial isolation and identification

The method used for the culture of *Salmonella* was according to the technique recommended by the International Organization for Standardization (Quinn et al., 1999; ISO-6579, 2002). The bacteriological media used in different stage were prepared according to the manufacturer's recommendations. The swab sample and a grinded tissue samples were transferred for pre-enrichment to buffered peptone water in the ratio of 1:9 and incubated for 24 ± 3 h at 37°C after that, 0.1 ml of the sample were selectively enriched in 10 ml of Rappaport Vassilliadis Soy Broth (RVS) and incubated aerobically at 41.5±1°C for 24 ± 3 h and then loop full of samples were platted out on xylose lysine deoxychlorate (XLD) medium incubated at 37°C for 20 to 24 h. Then *Salmonella* suspected colonies were examined for the presence of typical red colony with black center in XLD medium. Suspected colonies were cultured on nutrient agar and confirmed by biochemical tests: TSI, Urease, Indole, lysine decarboxylase and Vogues proskour tests. At this level, the genus of *Salmonella* was identified and suspected colonies were cultured on Biology Universal Growth (BUG) media for further species and subspecies confirmation.

For species and sub-species identification, OMNILOG (fully automated coated microplate based bacterial identification system) that is, GEN III microplate with protocol A method was used to test suspected colonies. A single colony grown on agar medium was selected and emulsified into 'inoculating fluid A' (IF A). According to the manufacturer's instructions, cell density of the bacterial inoculum was measured for a specified transmittance (90 to 98%) using a turbidimeter, as specified in the user guide. For each isolate, 100 µl of the cell suspension was inoculated in to each of the 96 well coated microplate, using automatic multichannel pipette and incubated aerobically at 33°C for 22 h. The OmniLog identification system automatically read each microplate and provide identification called species/sub-species ID, then the results were printed. The results were also read in the BIOLOG Micro Station reader after 22 h incubation outside GEN III incubator.

Data management and analysis

The Data were entered into Microsoft Office Excel spread sheets and was analyzed using STATA (version 12) statistical soft ware package. Descriptive statistics was used to determine the prevalence of salmonellosis in the study area. The association of infection with the different factors was analyzed using Chi-square test. A P-value less than 0.05 at 95% confidence interval was considered for significance.

RESULTS

From a total of 122 animals examined, 21 (17.21%) were positive for Salmonella; 9.83% (n=12) and 7.38% (n=9) were positive sheep and goats, respectively. There was significant difference (P < 0.05) in the frequency of *Salmonella* isolation between sheep and goat (Table 1).

From Luna and Elfora abattoirs, a total of 525 samples, 488 tissues and 37 pooled knives were collected. From this out of the 257 samples collected from Luna abattoir, 10 (3.89%) were *Salmonella* positive, and out of the 268 samples collected from Elfora abattoir *Salmonella* was detected in 11 (4.10%) samples. However, there was no significant difference (P > 0.05) in the frequency of *Salmonella* isolation between these two abattoirs (Table 2).

Salmonella was isolated from tissue samples collected from mesenteric lymph nodes (6.56%), cecum (5.73%), liver (4.09%) and abdominal muscles (0.82%). There was no significant difference (P > 0.05) in the frequency of *Salmonella* isolation among tissue samples (Table 3).

Salmonella sub species isolation

A total of 21 *Salmonella* isolates, consisting of two different subspecies were identified. Of the sub species identified during study, 20 were *S. enterica* subspp. *enterica* and 1 was *S. enterica* subspp. *salamae* (Table 4).

DISCUSSION

In the present study, from the total of 122 animals examined, 21 (17.21%) were positive for *Salmonella* of which 12(9.83%) were sheep and 9 (7.38%) were goats. The prevalence of *Salmonella* was higher in sheep than goats. This difference was statistically significant (P=0.04). This variation in prevalence of *Salmonella* between the two species might be due to differences in feeding behavior (sheep prefer to graze while goat to browse) and rearing area as well as management differences in the two species (Wassie, 2004). The higher prevalence in sheep might be due to higher *Salmonella* carrier rate in the study population. In addition, the sheep involved in this study came from different parts of the country by different means of transport and were usually held for a day to week before slaughter. The close contact during the transport and holding time may account for the high prevalence of *Salmonella* when examined after slaughter (Hurd et al., 2002; Molla et al., 2003).

D'Aoust (1989) cited few studies on the prevalence of *Salmonella* on sheep and goats undertaken in different parts of the world ranging between 2 and 51.5% in sheep and 1 to 18.8% in goats. Therefore, the finding in the present study was in line with reports of D' Aoust (1989). However, the same author reported a prevalence of 14.7% in sheep which was lower than the current finding (D'Aoust, 1994). This might be due to the fact that animals had been held in the market for longer period before slaughtered where stress could contribute to the

Table 1. Prevalence of *Salmonella* in slaughtered sheep and goats.

Species	No. examined	Positive	Prevalence within species (%)	Prevalence from the total (%)	χ^2	P-value
Ovine	44	12	27.27	9.83		
Caprine	78	9	11.54	7.38	4.88	0.027
Total	122	21	17.21	-		

Table 2. Prevalence of *Salmonella* between Luna and Elfora abattoirs.

Sample type	Number of samples from				Total	
	Luna abattoir		Elfora abattoir			
	Examined	Positive (%)	Examined	Positive (%)	Examined	Positive (%)
Cecum	60	2 (3.33)	62	5 (8.06)	122	7 (5.73)
Liver	60	4 (6.67)	62	1 (1.61)	122	5 (4.09)
Mesenteric lymph node	60	3 (5.0)	62	5 (8.06)	122	8 (6.56)
Abdominal muscle	60	1 (1.67)	62	0 (0.0)	122	1 (0.82)
Pooled knife	17	0 (0.0)	20	0 (0.0)	37	0 (0.0)
Total	257	10 (3.89)	268	11 (4.10)	525	21 (4.0)

Table 3. Risk factors for isolation of salmonella from apparently healthy slaughtered sheep and goats

Risk factors	Examined	Positive (%)	χ^2	P. value
Abattoirs				
Elfora	248	11 (4.4)	0.021	0.884
Luna	240	10 (4.2)		
Species				
Goat	312	9 (2.9)	4.23	0.04
Sheep	176	12 (6.8)		
Tissues				
Abdominal Muscle	122	1 (0.8)	5.722	0.13
Cecum	122	7 (5.7)		
Liver	122	5 (4.1)		
Mesenteric Lymph nodes	122	8 (6.6)		
Total	488	21 (4.3)		

Table 4. Distributions of identified *Salmonella* subspecies by animal species and abattoir sources.

Identified ***subspecies***	Luna		Elfora		Total of the two animal species		
	Sheep	Goat	Sheep	Goat	Sheep	Goat	Total
S. enterica subspecies enterica	3	7	8	2	11	9	20
S. enterica subspecies salamae	0	0	1	0	1	0	1
Total	3	7	9	2	12	9	21

higher infection rate among the animals (Teklu and Negussie, 2011). This holds true for small ruminants slaughtered at Luna and Elfora export abattoir, where the animals stayed for up to a week before slaughtered, especially at Elfora export abattoir when there was scarcity of animal supply from the customer.

The overall prevalence of the current result was higher than previous findings by different researchers in different parts of the country and elsewhere; in which Sierra et al. (1995) reported 10% prevalence from freshly dressed carcasses in Spain, Woldemariam et al. (2005) reported 2.8% prevalence in Debrezeit and Wassie (2004) reported prevalence of 11.3% in Addis Ababa and Modjo abattoirs. Similarly, Teklu and Negussie (2011) had also reported prevalence of 7.7% in Modjo export abattoirs and Zubair and Ibrahim (2012) reported prevalence of 2.5% from Zakho abattoir, Kurdistan region, Iraq.

The prevalence of *Salmonella* in apparently healthy slaughtered goats in this study was 11.54%. This results fall in the range of 1 to 18.8% prevalence in goats from different countries (D'Aoust, 1989). This finding was also in line with the findings of Teklu and Negussie (2011), who reported 11.7% in Modjo, Ethiopia and Woldemariam et al. (2005), who reported 9.8% in apparently healthy slaughtered sheep and goats in Bishoftu Ethiopia. However, the prevalence in goats was higher than prevalence reported by Wassie (2004) in Addis Ababa abattoir, which was 3%, Zubair and Ibrahim (2012), 2% from Zakho abattoir, Kurdistan region, Iraq, and Bedaso et al. (2015) who reported 0.54% from apparently healthy goats and sheep at Addis Ababa abattoir enterprise, Ethiopia. Similarly Sharma et al. (2001) had also reported prevalence of 2.3% from goats samples in Zambia. Even though, the current prevalence was higher than what was discussed, it was also lower than that in Ferede et al. (2015) in which it has 17.7% was reported from apparently healthy goats at Dire Dawa municipal abattoir, and 16.7% prevalence reported from goats slaughtered at Elfora abattoir in Ethiopia (Woldemariam, 2003). This variation in reported prevalence could be associated with the sampling plan and procedures, sample type, bacteriological techniques employed in detecting *Salmonella* or difference in occurrence and distribution of *Salmonella* in the study population regardless of test samples and methods of detection and hygienic condition of the abattoir environment.

In this study, *Salmonella* was not found from 37 pooled samples of knife swab both at Luna and Elfora export abattoirs. This finding was contrary to results of Teklu and Negussie (2011), who had reported 7.4% *Salmonella* prevalence from eviscerating knife swabs and 5% prevalence report of eviscerating knives in poultry slaughter houses in Iraq Sultan and Sharif (2002). Moreover, other study on knife blades had also reported 26.7 and 10% prevalence in two Botswana abattoirs (Motsoela et al., 2002). The difference between the results of the previous and the present one could be due to improvement of the hygienic conditions of the knife. Moreover, immersion of knives in hot water is being practiced in both abattoirs after flaying each animal and this might have also resulted in the low contamination of abdominal muscle observed in both abattoirs.

From a total of 488 examined sheep and goat tissue samples, 4.3% were infected with *Salmonella*. Of the four tissue samples taken from each animal during the study period, the cecum, liver and mesenteric lymph node samples proved to be the most useful indicators of infection. Abdominal muscle samples were less infected and this result was also similar to the findings of Molla et al. (2006).

Salmonella isolation rate of 11.36% that was recorded from cecum samples of sheep in this study was higher than the earlier observation of 2.1% by Teklu and Negussie (2011), 4.8% by Wassie (2004) in feaces, 2.1% report of Woldemariam (2003) from feaces and 6.7% reported by Bedaso et al. (2015). The 2.56% prevalence in goat cecum in this study was comparable to that of 3.3% by Woldemariam (2003) from feaces and 2% by Wassie (2004) from feaces. Other researchers have also reported the presence of human pathogens, such as *Salmonella*, in animal feaces (Jiang et al., 2015). The higher isolation rate from cecum in this study clearly indicates that *Salmonella* is found in the cecum microflora. Usually, healthy carriers intermittently excrete only a few *Salmonella*, unless they undergo some kind of stress (example during transport or holding in the lairages prior to slaughter).

The current study revealed that the isolation rate of *Salmonella* from mesenteric lymph node in sheep was 13.6% and in goats was 2.56%. This finding was close to 8.1% finding of Pateraki et al. (1975) in Greece and 7% report of Tadesse et al. (2014) from Adama municipal abattoir, Ethiopia. High level of *Salmonella* isolation in mesenteric lymph nodes in current finding may be due to the animal stay for up to a week before slaughter. However, study conducted on apparently healthy slaughtered sheep in Australia by Moo et al. (1980) indicated a 4% prevalence of *Salmonella* in mesenteric lymph nodes that was lower than the current finding.

From this study *Salmonella* isolation rate from liver of sheep was 2.27%. This finding was low in relation to isolate found in mesenteric lymph nodes and ceacal content. Low isolation rates in liver of sheep in this study support the findings of Molla et al. (2006) 1.9%, and Wassie (2004) 1.9%. However, Bedaso et al. (2015) and Tadesse et al. (2014) reported that there was no *Salmonella* isolate found in the liver. The low detection rates in these organs indicate that localization of the organism in liver is most likely minimal. It appeared to be rare for the liver and spleen tissue to be infected with *Salmonella* before death Molla et al. (2006). In goat, the finding was higher than sheep that was 5.13%. In contrast to this result, no *Salmonella* isolate was reported by Molla et al. (2006) in goat. This variation in result may be due to cross contamination during sampling or due to different bacteriological procedures followed.

In the present study, *Salmonella* was isolated in 1.28% of the abdominal muscle of goat but not detected from the sheep' abdominal muscle. This finding is much lower

than the 10% report from freshly dressed carcasses of sheep in Spain (Sierra et al., 1995), 17.7% from goat carcass swab in Dire Dawa municipal abattoir in Ethiopia (Ferede et al., 2015). However, the present finding was in consistence with Molla et al. (2006). It is generally accepted that the carcass of healthy slaughtered animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion (Jay, 2000). These differences in prevalence of abdominal muscle may be due to hygienic condition of abattoirs and its environment. The current study revealed that as the carcass contaminations of the study area were low and this indicates that they were found at good hygienic condition.

Out of the total 21 *Salmonella* isolates, two different *Salmonella* subspecies were identified, this were *S. enterica* subspp. *enterica* 20/21 and *S. enterica* subspp. *salamae* 1/21. The current results indicate that the pathogenic and zoonotic *S. enterica* subspp. *enterica* was highly prevalent compared to the non-pathogenic *S. enterica* subspp. *salamae.* Hence, 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 that is from farm to fork. Therefore, during evisceration the offal content and carcass should not be in contact to each other and immediate separation of the offal from the carcasses should be employed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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