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Short Communication

Prevalence of *Salmonella* on Sheep Carcasses Slaughtered at Adama Municipal Abattoir, South Eastern Ethiopia

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Abstract

A cross-sectional study was carried to determine the prevalence of *Salmonella* and *Salmonella* Serogroups from cattle carcasses slaughtered during November 2011 to April 2012 at Adama municipal abattoir. From a total of 100 sheep carcasses, 300 samples (abomasums, liver and mesenteric lymph nodes) were collected aseptically. Out of 100 animals examined sheep, 14 (14.0%) were *Salmonella* carriers. Whereas among 300 examined sheep carcass, *Salmonella* was 17 (5.6%) were found to be positive based on culture methods and different biochemical tests that were employed. The *Salmonella* was detected in 13 (13%), 7 (7%) of each 100 samples of abomasum's and mesenteric lymph nodes respectively. Out of the 17 *Salmonella* isolates, three different Serogroups were identified of which Serogroup B was predominant (63.6%) followed by Serogroup C_2 (21.2%) and Serogroup D (15.2%). Results of the present study indicated that *Salmonella* is a common problem in slaughtered sheep carcasses in Adama municipal abattoir. Therefore, Sources of pathogens in food animals need to be investigated and a further study of pathogens in the food chain is recommended.

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INTRODUCTION

Salmonella is a leading cause of food borne illness (WHO, 1998; White *et al*., 2001). Globally, more than 93 million cases of gastroenteritis are caused by non typhoidal Salmonella with 155,000 deaths each year. Of these cases, 80.3 million cases were estimated to be food borne. The diseases caused by bacteria of the genus Salmonella, is a common intestinal illness caused by numerous Salmonella serovars with clinical manifestations that vary from severe enteric fever to mild food poisoning both in animals (Radostits *et al*., 2007) and humans (Hohmann, 2001). Foods of animal origin particularly meat, poultry, egg, milk and milk products are considered to be the primary source of human salmonellosis (Acha and Szyfers, 2001). Most of these food products become contaminated during slaughter, processing in contaminated environment and because of faulty in transport, handling, storage or preparation. Salmonellosis takes a healthy tool in human life and suffering, particularly among infants and children, the elderly and other susceptible persons particularly in developing countries. According to the World Health Organization reports of 1995, 88% of all food borne disease was caused by *Salmonella*. Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species i.e. *Salmonella enterica* and *S. bongori*. *Salmonella enterica*, a Gram-negative, non-sporing, catalase-positive, oxidase-negative facultative anaerobic bacillus is a significant cause of morbidity and mortality in humans and animals, with multidrug-resistant *S. enteric* serovar *typhimurium* being an emerging problem (Gabert *et al*., 1999).

Salmonella species have been also extensively incriminated worldwide as common causes of bacterial gastroenteritis in humans, with food-animals serving as important reservoirs (Acha and Szyfres, 2001). The epidemiology of food borne problems like salmonellosis is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers and consumers, demographic changes (increased susceptible population), international travel and migration, international trade in food, animal feed and in animals, poverty and lack of safe food preparation facilities (Van der Venter, 1999; Altekruse *et al*., 1998).

Food borne illnesses, including salmonellosis, are widespread and have an impact on communities in both the developing and developed world. In industrialized countries the incidence of salmonellosis is on the rise due to the emergence and increase of *S. enteritidis* and *S. typhimurium* DT 104 (Wray and Davies, 2000). Hundreds of millions of people worldwide suffer from communicable and non-communicable diseases caused by contaminated food. These diseases take a heavy toll in human life and sufferings, particularly among infants and children, the

elderly and other susceptible persons. They also create an enormous social, cultural and economic burden on communities and their health system. Interest in Salmonella has heightened in recent years due to the increased susceptibility of AIDS patients to salmonellosis, the devastating effects of S. Enteritidis in the poultry industry, and the globalization of agricultural trade. Persistent and severe salmonellosis has also been recognized as a problem among patients with AIDS (Clarke and Gyles, 1993).

Contaminated food of animal origin, particularly meat products are also an important source of *S. typhimurium* in human infections. *S. typhimurium* has been described as a collection of variants that vary significantly in their host range and their degree of host adaptation. It is the third most common serovar causing human food-poisoning in some parts of the world (Alemayehu *et al*., 2002). The disease can affect all species of domestic animals; however, young animals and pregnant animals are the most susceptible. Enteric disease, often presenting as bloody or profuse watery diarrhea with pyrexia, is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicemia, abortion, arthritis, necrosis of extremities and respiratory disease, may be seen. The signs and lesions are not pathognomonic. Many animals, especially poultry and pigs, may also be infected but show no clinical illness (Wray and Davies, 2000).

The true incidence of salmonellosis both in humans and animals is difficult to evaluate because of lack of an epidemiological surveillance system in place, which is particularly true in developing countries. However, the number of outbreaks particularly in humans following consumption of contaminated meat of sheep and goat origin has increased considerably in recent years. Carrier states of humans are of concern to the food manufacturing and food service industries because of the perceived risk of contamination of food by infected food handlers and the risk of food borne disease outbreaks (Mache, 2002). The presence of even small numbers of *Salmonella* in carcass meat and edible offal's may lead to heavy contamination of the already processed and packed meat for distribution. When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue. Raw meats, particularly minced meats have very high total counts of microorganisms and salmonellae are likely to be present in large numbers. Hence, monitoring the prevalence of *salmonellae* at abattoirs is imperative for designing of effective prevention and control of such pathogens before they further enter the food chain (Molla and Mesfin, 2003). Thus, the objective of the present study was to determine the prevalence of S*almonella* and identify the *Salmonella* serogroups isolated on slaughtered sheep at Adama municipal abattoir.

MATERIALS AND METHODS

Study Area

The study was conducted in Oromia regional state, East shewa zone at Adama City which is found about 99 km south eastern of Addis Ababa, the capital city of Ethiopia. The City is located at $08^0$33N $39^0$16E. The Ethio-Djbouti Rail way that crosses the city and about 300,000 people are live in it. Moreover, about 36,000 estimated people visit every day (CSA, 2009). The populations of the city are increasing from time to time so that the demand of meat consumption is rising from time to time. Adama Municipal Abattoir is located at Boku shenan kebele on the main road of Adama-Wanji road. In this abattoir an average 20 sheep are slaughtered every day.

Study Animals

Animal population for this study was sheep brought to the abattoir from different areas mainly from Arsi, Harar, Bale and Borena. The study was conducted on organ samples derived from slaughtered sheep carcasses at the abattoir.

Study Design

A cross sectional study was conducted from November 2011 to April 2012 to determine the prevalence of salmonellosis in sheep carcass slaughtered at Adama municipal abattoir.

Sample Size

The sample size required for this study was determined depending on the expected prevalence of *Salmonella* and the desired absolute precision according to Thrusfield (2007). It is possible to take 50% expected prevalence. The Z value of 1.96 is used at 95% CI and margin of error is 5% (n = sample size, P=proportion, d= margin of error).

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

Where, n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision.

Study Methodology

Samples were collected aseptically using sterile scalpel blade to cut the desired amount of tissue samples (25g) and sterile plastic bag was used to hold the samples from slaughtered sheep carcasses during slaughtering operations. Sample was collected from purposely selected animals showing characteristic lesions like enlarged fatty and congested liver, inflamed abomasum and enlarged, moist or bleeding mesenteric lymph nodes (three types of samples from each carcass: liver, abomasum and mesenteric lymph nodes) were collected once in a week and a total of 300 samples were collected from 100 Sheep carcasses slaughtered during the study period. The connective tissue and fat were trimmed from the mesenteric lymph nodes before mixing and pre-enrichment. Samples in sterile containers were put in an icebox and transported immediately to the food microbiology laboratory of the Aklilu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia for further processing and laboratory analysis.

Culture Methods

Isolation and identification of S*almonella* was performed following the techniques recommended by the International Organization for Standardization (Quinn *et al.,* 1994; ISO 6579, 1998). Twenty-five gram of each sample was weighed and organ samples were cut into smaller fine pieces using sterile scalpel blades. The prepared sample was put in a stomacher bag and to each of the 25 gm sample; 225 ml of buffered peptone water (BPW) (Park, Northampton, UK) was added. An amount of 9ml of BPW for each gram of sample (1:9 sample weights to BPW volume ratio) was used for pre

enrichment of samples, which were below 25 gm. Each sample in the stomacher bag with the pre-enrichment medium (BPW) was macerated using a stomacher (Seward Stomacher 400, London) for 2 min at high speed and then incubated at 37 ^{0}C for 16-20 hrs.

A 0.1 ml of the pre-enrichment broth was transferred aseptically to 10 ml of Rappaport–Vassiliadis (RV) soya broth (Oxoid, England) and incubated for 18–24 hrs at 42 ^{0}C. A loop full of each enrichment broth was streaked onto xylose lysine deoxycholate agar (XLD) (Fluka, Spain) and incubated at 37 ^{0}C for 24 hrs. The plates were then examined for the presence of *Salmonella* colonies. Putative *Salmonella* colonies were sub cultured onto nutrient broth (Fluka, Switzerland) and subjected to different biochemical tests following standard methods (Quinn *et al.,* 1994; ISO 6579, 1998).

Biochemical Tests

All suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, lysine iron agar, Simmon's citrate agar, urea agar and peptone water (indole) and incubated for 24 or 48 hours at 37^{o}C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide and gas production, positive for lysine (purple color), negative for urea hydrolysis (remained unchanged), negative for tryptophan utilization (indole test) (yellow-brown ring), and positive for citrate utilization (blue color) were considered to be *Salmonella*-positive (Quinn *et al.,* 1994; ISO 6579, 1998).

Serology

Positive colonies of putative *Salmonella* organisms were further tested for agglutination by rapid slide agglutination test using *Salmonella* polyvalent antisera set 1 (Poly O, Poly O1 and VI) (Mast diagnostics, Mast group Ltd, Merseyside, UK) in accordance with the manufacturer's instructions and ISO 6579 (1998) recommendations. Briefly, some bacterial mass from suspected colonies grown on nutrient agar was mixed with a drop of sterile saline solution and it was emulsified to produce a distinct uniform turbidity then a drop of polyvalent antiserum O was added on to the emulsified isolates and the reagents were mixed by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background, distinct clumping or agglutination within this period was observed and regarded as positive result and weak agglutination was regarded as negative. In order to exclude any spontaneous agglutination (auto agglutination) a negative control using physiological saline solution and bacterial colony to be tested was done. *Salmonella* polyvalent antiserum O_1 was used when suspected bacteria fail to agglutinate with *Salmonella* polyvalent O antiserum I.

Data Analysis

The data obtained from the abattoir and the laboratory analysis result were recorded and entered into SPSS version 21.0 and summarized using descriptive statistical methods (percentage, table).

RESULTS

Prevalence and Distribution

Out of the total 100 animal carcass examined, 14 (14.0%) were *salmonella* carriers. Out of the total 300 samples from 100 animals examined, *salmonella* was isolated in 17 samples representing 5.60% samples of sheep carcass were *salmonella* positive based on culture methods and different biochemical tests employed. *Salmonella* were isolated from two types of the test samples (abomasum and mesenteric lymph node) with different frequencies of occurrence. However, liver samples were found to be *salmonella* negative. There is a significant difference in the distribution of *salmonella* isolates among the sample type in which the highest proportion 13 (13%) was isolated from abomasum followed by 7 (7%) of the mesenteric lymph node samples (Table 1).

Table1: Prevalence and distribution of *salmonella* from sample sources

Sample type	**Number of Samples**	
	Sheep (n=100) Positive= 14%	
	Total Examined	**Positive (%)**
Liver	100	-
Abomasum	100	13 (13%)
Mesenteric lymph node	100	7(7%)
Total	300	17 (17%)

Serotyping

Out of the total 17 *salmonella* isolates, three different seogroups were identified with different frequencies of occurrence. The most frequent serogroup was serogroup B (82.4%), followed by serogroup C_2 and serogroup D (each 11.1%) respectively. The highest proportion (55.5%) of Serogroup B was identified from abomasum samples as compared to mesenteric lymph node and liver (each 22.2% and 0%) respectively. The distribution of serogroups in the various tissue samples showed that serogroup B was the most prevalent in which out of nine positive *salmonella* samples, serogroup B was detected in seven (5 abomasum and 2 mesenteric lymph node) samples. *Salmonella* serogroup C_2 (12.0%) and D (7.60%) were detected only from one mesenteric lymph node and one abomasum samples respectively and either of those serogroups were not detected in liver (Table 2).

Table 2: Distribution of *Salmonella* Serogroups isolated from slaughtered sheep carcasses at Adama Municiplal abattoir

***Salmonella* Serogroups**	**Liver**	**Abomasum**	**Mesenteric Lymph Node**	**Number of Serogroups**	**Total (%)**
B	-	15	6	21	**21 (63.6%)**
C_2	-	-	7	7	**7 (21.2%)**
D	-	5	-	5	**5 (15.2%)**
Total	**-**	**20**	**13**	**33**	**33 (100%)**

DISCUSSION

In the present study, the overall prevalence of *Salmonella* in sheep carcass slaughtered at Adama municipal abattoir was found to be 14.0%. D'Aoust, (1989) reported a prevalence of *Salmonella* to range between 2 and 51.5% in sheep. Nabbut and Al-Nakhli (1982) also reported a prevalence of 14.7% in apparently healthy slaughtered sheep in Riyadh public abattoir, Saudi Arabia. The prevalence of the present study is relatively higher than similar study reported by Woldemariam *et al.* (2004) who reported 2.80% prevalence in sheep slaughtered at Debre Zeit abattoir. However, it is in line with the result of Molla *et al*. (2006) who reported an overall prevalence of 11.5% in apparently healthy slaughtered sheep of central Ethiopia. Similar study conducted by Wassie, (2004) also reported the same result as Molla *et al*. (2006) reported a prevalence of 11.5% in slaughtered sheep. Ejeta *et al.* (2004) also indicated a prevalence of 14.7% in minced beef, mutton and pork at the same abattoir. High prevalence of salmonellosis in different food animals and meat products are again reported by Molla and Mesfin (2003); Molla and Alemayehu (2003); Molla, Kleer and Sinell (1999).

The difference in the reported prevalence could be associated with the sampling plan and procedures, sample type, distribution of *salmonella* in a lot examined and the method of detection employed (Woldemariam *et al*., 2004). It is also known that keeping animals to be slaughtered in the abattoir's waiting pens crowded could facilitate the excretion and transmission of infection among the animals. In addition to this, stress could induce higher infection rates among animals when they are held in the market for longer period before slaughter.

Of the sample types taken from each animal during the study period, the mesenteric lymph nodes and abomasum samples proved to be useful indicators of infection, as most of the sheep and all *Salmonella* positive sheep were detected on the basis of those samples. The tissue prevalence distribution of *Salmonella* isolate was 7% in mesenteric lymph node. The result is in agreement with the finding of Molla *et al*. (2006) who reported 8.7% and 6.9% prevalence in mesenteric lymph node at Addis Ababa and Modjo abattoir respectively. The findings in the mesenteric lymph nodes of sheep was also supported by earlier observation by Akafete and Haileleul (2011) who reported 5.6% prevalence in mesenteric lymph nodes in slaughtered sheep at Modjo export abattoir. However, the result is lower than the finding of Woldemariam *et al.* (2004) who reported 11.7% prevalence in mesenteric lymph node at Debre-Zeit ELFORA abattoir. The result is also slightly less than the finding of Wray and Davies (2004) who indicated 7.7% in mesenteric lymph nodes. The present finding was also in agreement with the study which was conducted in apparently healthy slaughtered sheep in Australia with 4% reported prevalence of *salmonella* in mesenteric lymph nodes (Molla *et al*., 1980).

The high proportion of infected sheep (7%) harbouring *salmonella* in their mesenteric lymph nodes might be associated with the existence of infection burden among *Salmonella* carrier animals and from animals acquiring new infection prior to slaughter due to different predisposing factors like starvation, overcrowding transportation and longer lairage confinement. The present high mesenteric lymph node prevalence of salmonella indicates the existence a substantial risk of cross-contamination during slaughtering, dressing and subsequent handling, of the carcasses (Molla *et al*., 2006). This indicated that the fecal contamination of the edible organs and carcass may occur during slaughtering operations unless hygienic measures are taken.

The prevalence of *Salmonella* was found to be 13% and significantly higher in abomasum ($P<0.05$). Identification of higher proportion of *Salmonella* in abomasum might be due to fecal material contamination and poor hygienic status of working personnel, slaughter house and equipments during slaughtering operation.

The present study also entails that liver samples did not appear to harbour *salmonella* on most occasions. Similar studies conducted elsewhere by Samuel *et al*. (1981) also reported that liver samples were found to be free of *Salmonella* organisms. This indicates that the organisms did not spread beyond the lymph nodes or if they did they were in numbers too small to be detected by the method used (Nabbut and Al-Nakhli, 1982). The low prevalence of *salmonella* in liver of sheep in this study supports other findings (Nabbut and Al-Nakhli, 1982), indicating that localization of the organisms in this organ is most likely minimal. The liver is usually free of *Salmonella* at slaughter, but the surfaces can become contaminated during processing. The ultimate source of this contamination is likely to be the *Salmonella* present in the gastrointestinal tract and mesenteric lymph nodes either of the same animal or of other animals slaughtered on the same day (Nabbut and Al-Nakhli, 1982).

In this study, *Salmonella* sero-group B was the most frequently isolated (63.6%) serovar followed by *Salmonella* serogroup C_2 (21.2%) and *Salmonella* serogroup D (15.2%). These serogroups were also reported by Molla *et al*. (1999) the predominant serotype reported by from minced beef from supermarkets was *S. dublin*. Nyeleti *et al.* (2000) reported *S. anatum* as a dominant serotype from minced beef samples collected from supermarkets in Addis Ababa. The more prevalent serotype (*S. braenderup*) reported by Ejeta *et al.* (2004) from mutton and pork which was not isolated on the study.

CONCLUSIONS

The present study indicated that *Salmonella* was prevalent in sheep carcasses in Adama municipal abattoir. Different serotype identified in this study reflects the possible cross-contamination from multiple sources at the slaughterhouse and poor hygiene during meat cutting and evisceration. *Salmonella* contamination was widespread in tissue samples of slaughtered sheep carcass at the abattoir and the magnitude of the problem is especially high in abomasum samples as compared to others which represent a real public health hazard. Thus, microbiological analysis of tissue samples from abattoir for food borne organisms like *Salmonella* is of paramount importance in ensuring the supply of safe and quality meat products for the consumers.

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