

Prevalence and Distribution of *Salmonella* in Slaughtered Sheep at Addis Ababa Municipal Abattoir, Ethiopia

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Abstract

Introduction: *Salmonella* is one of the most significant enteric pathogen from food safety point of view in which it is classified among the major cause of food borne illnesses.

Objective: To determine the prevalence of *Salmonella* in slaughtered sheep and to identify underlying serogroups from slaughtered sheep at Addis Ababa municipal abattoir, Ethiopia.

Methods: A cross-sectional study was conducted to identify *salmonella* serogroups. Tissue samples; liver, abomasums and mesenteric lymph node were collected aseptically using sterile scalpel blade in a sterile plastic bag. Isolation and identification of *Salmonella* was performed following the techniques recommended by the International Organization for Standardization 6579. Prevalence was calculated and serotyping was done using rapid slide agglutination test.

Results: Of 50 animals examined, one or more of the samples were *Salmonella* positive in 8 (5.33%) of the animals and from a total of tissue samples examined, 9 (6%) were *Salmonella* positive. Of the nine *Salmonella* isolates, three different serogroups were identified of which serogroup B (O4) was predominant (77.8%) followed by Serogroup C2 (O8) (11.1%) and Serogroup D (O9) (11.1%).

Conclusion: *Salmonella* was widespread in tissue samples of slaughtered sheep carcasses in the Addis Ababa abattoir. Sources of pathogens in food animals need to be investigated and further study of *salmonella* and other enteric pathogens in the public food chain is recommended. Also their food safety implication has to be further evaluated.

Keywords: Prevalence; *Salmonella*; Sheep carcasses

Introduction

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-spore, catalase-positive, oxidase-negative facultative anaerobic bacillus is a significant cause of morbidity and mortality in humans and animals, with multidrug-resistant *S. enterica* serovar *typhimurium* being an emerging problem (Gabert et al. 1999). Numerous *Salmonella* serovars cause acute and chronic enteritis, acute septicemia or subclinical infections in animals and humans. *Salmonella* species have also been 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 the disease 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, international travel and migration, international trade in food, animal feed and in animals and poverty and lack of safe food preparation facilities (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). 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). Recent studies (DiMarzio et al. 2013) also showed that in the developed countries, the number of antibiotic-resistant isolates identified in humans is steadily increasing, suggesting that the spread of antibiotic resistant strains is a major threat to public health. 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; young animals and pregnant animals are the most susceptible. Many animals especially, poultry and pigs could be infected but show no clinical illness (Wray and Davies 2000).

Human carriers of the pathogen 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 et al. 1997).

The presence of even small numbers of *Salmonella* in carcass meat and edible offal 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 *Salmonella* are likely to be present in large numbers. Hence, monitoring the prevalence of *Salmonella* at abattoirs is imperative for creating a data bank and for effective control of such pathogens before they further enter in to the food chain (Molla and Mesfin 2003).

Materials and methods

Study design and Sample Collection: A cross sectional study design was used for the determination of *Salmonella* prevalence in organ samples derived from slaughtered sheep at Addis Ababa municipal abattoir from October 2013 to March, 2014. 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 slaughtered 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 150 samples were collected from 50 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 EPHI, Addis Ababa, for further processing and laboratory analysis.

Isolation and identification of *Salmonella*

Culture Methods

Isolation and identification of *Salmonella* was performed following the techniques recommended by the International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994). 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 °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 °C. A loop full of each enrichment broth was streaked onto xylose lysine deoxycholate agar (XLD) (Fluka, Spain) and incubated at 37 °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 of International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994).

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°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 (International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994).

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). Suspected colonies grown on nutrient agar were 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 and then reagents were mixed by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background, it was considered as positive when agglutination was observed, and negative if no agglutination.

To exclude any spontaneous agglutination, a negative control using physiological saline solution and bacterial colony was done. *Salmonella* polyvalent antiserum O₁ was used when suspected bacteria fail to agglutinate with *Salmonella* polyvalent O antiserum I. Data was summarized using descriptive statistical methods.

Results

Prevalence and Distribution

Of the total 150 samples from 50 animals examined, *salmonella* was isolated in 9 (6%) samples of 8(16.0%) sheep carcasses. Based on culture methods and biochemical tests employed, *Salmonella* were

isolated from abomasum and mesenteric lymph node samples with the highest proportion 6 (12%) from abomasum and 3 (6%) from the mesenteric lymph node. All liver samples were found to be *Salmonella* negative (Table 1).

Table 1: Prevalence and Distribution of salmonella from sample sources

Sample type	Total no. examined	Positive no. (%)
Liver	50	-
Abomasum	50	6 (12)
Mesenteric lymph node	50	3 (6)
Total	150	9 (6)

Serotyping

Out of the total nine (9) *Salmonella* isolates, three (3) serogroups were identified. Serogroup B was the predominant (77.8%), followed by serogroup C₂ and serogroup D (each 11.1%). The highest proportion (55.5%) of Serogroup B was identified from abomasum samples as compared to mesenteric lymph node (22.2%). 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 C₂ (11.0%) and D (11.0%) were detected only from one mesenteric lymph node and one abomasum samples respectively (Table 2).

Table 2: Distribution of Salmonella serogroups isolated from slaughtered sheep carcasses.

<i>Salmonella</i> Serogroup	Abomasum	Mesenteric lymph node	Total no. (%)
B (O4)	5	2	7 (77.8)
C ₂ (O8)	-	1	1 (11.1)
D (O9)	1	-	1 (11.1)
Total	6	3	9 (100)

Discussion

The prevalence of *Salmonella* in slaughtered sheep in this study was found to be 16%. This is in line with studies done in central Ethiopia by Molla et al. (2006) and Wassie (2004) which was 11.5% and another study done in in Riyadh, Saudi Arabia (14.7%) (Nabbut and Al-Nakhli 1982) but relatively higher than a study done in Bishoftu abattoir by Woldemariam et al. (2005) which was 2.8%. The difference in the reported prevalence could be associated with the sampling procedures, sample type, distribution of *salmonella* in a lot examined and the method of detection employed (White et al. 2001). It is also known that keeping animals to be slaughtered in the abattoir's waiting pens crowded could facilitate the transmission of infection among the animals. 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 6% in mesenteric lymph node which is in agreement with the finding of Molla et al. (2006) who reported 8.7% and 6.9% prevalence

at Addis Ababa and Modjo abattoirs respectively. This finding is also supported by earlier observation by (Akafete and Haileleul 2011) who reported 5.6% prevalence at modjo export abattoir. However, the result is lower than the finding of Woldemariam et al. (2005) who reported 11.7% prevalence in mesenteric lymph node at Debre-Zeit ELFORA abattoir and slightly less than the finding of Wassie (2004) who indicated 7.7% at Addis Ababa and Mojo abattoirs. It is also consistent with the study conducted in apparently healthy slaughtered sheep in Australia (4%) in mesenteric lymph nodes (Moo et al. 1980).

The findings of a high proportion of infected sheep (6%) harboring *Salmonella* in their mesenteric lymph nodes might be associated with the existence of high 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* also indicates the existence of a substantial risk of cross-contamination during slaughtering, dressing and subsequent handling of the carcasses where fecal material contamination of the edible organs and

carcass may occur during slaughtering operations unless hygienic measures are taken (Molla et al. 2006).

The prevalence of *Salmonella* in abomasum was found to be 12% which was significantly higher ($p < 0.05$). Identification of higher proportion of *Salmonella* in abomasum might be due to fecal material contamination and poor hygienic status of working personnel, slaughterhouse and equipment during slaughtering operation. The present study also entails that liver samples did not appear to harbor *salmonella* on most occasions which is similar with studies conducted by Samuel et al. (1981), Nabbut and Al-Nakhli (1982) and Nyeleti et al. (2000). This indicates that the organisms did not spread beyond the lymph nodes or if they did, they were too small in numbers to be detected by the method used; the liver is usually free of *Salmonella* at slaughter, but surfaces can be contaminated during processing. The ultimate source of this contamination is likely to be the *Salmonella* present in the gastrointestinal tract and mesenteric lymph nodes of the same animal or other animals slaughtered on the same day (Samuel et al. 1981; Nabbut and Al-Nakhli 1982).

In the study *Salmonella* serogroup B (O4) (77.8%) was the most frequently isolated serogroup followed by *Salmonella* serogroup C2 (O8) (11.1%) and *Salmonella* serogroup D (O9) (11.1%) from sample sources. All of those serogroups were detected by Molla et al. (2003) from food animals, slaughter house personnel and retail meat products in Ethiopia. Serogroup B and C2 were also reported by Ejeta et al. (2004) in mutton from retail supermarkets in Addis Ababa, Ethiopia. Another similar study conducted by Molla et al. (2006) indicated that serogroup B and D were most prevalent in apparently healthy slaughtered sheep of central Ethiopia. Serogroup B was also reported by Woldemariam et al. (2005) in apparently healthy slaughtered sheep in Bishoftu. Serogroup B was the major serogroup among the present isolates in which it contains certain pathogenic serotypes like *S.typhimurium*, *S. enteritidis* and *S. Dublin* and others. The detection of all those serogroups which probably contains those pathogenic serotypes in apparently healthy slaughtered sheep is of paramount health significance since contaminated edible organs may pose serious public health risks.

Conclusion

The present study indicated that *Salmonella* was prevalent in sheep carcasses in Addis Ababa municipal abattoir. Different serogroups 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, sources of pathogens in food animals need to be investigated and further study of pathogens in the food chain is also needed.

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References

- AACSA (Central Statistical Agency of Federal Democratic Republic of Ethiopia, 2009.
- Acha PN & Szyfres B (2001). Zoonosis and Communicable Diseases Common to Man and Animals. 3rd eds., Washington DC: Pan American Health Organization, 2001; 233-246.
- Akafete T & Haileluel N (2011). Assessments of Risk factors and Prevalence of *Salmonella* in Slaughtered Small Ruminants and Environment in An Export Abattoir, Modjo, Ethiopia. *American-European Journal of Agriculture and Environmental Science*, 10(6): 992-996.
- Alemayehu D, Molla B & Muckle A (2002). Prevalence and antimicrobial resistance of *Salmonella* isolated from apparently healthy slaughtered cattle in Ethiopia. *Tropical Animal Health and Production*, 35, 309-316.
- Altekruse SF, Swerdlow DL & Wells SJ (1998). Factors in the emergence of foodborne diseases. *Vet. Clin. North Amer. Food Animal Practice*, 14: 1-15.
- Clarke RC & Gyles CL (1993). *Salmonella*. In: Gyles CL & CO. Thoen. (eds.) *Pathogenesis of Bacterial Infections in Animals*. 2nd ed. Ames, IA: Iowa State University. 133-153.
- D'Aoust JY (1989). *Salmonella*. In Doyle MP. (ed). *Food-borne Bacterial Pathogens*. New York Marcel Dekker Inc. 327-445.
- Ejeta G, Molla B, Alemayehu D & Muckle A (2004). *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue de Médecine Vétérinaire*, 155 (11): 547-551.
- Galbert J, Schalch B, Greil B, Sperner B, Stollw A & Weber C (1999). The use of a commercial test system (SALMOTYPE®-ELISA) for tracing antibodies against *Salmonella* in the serum of pigs. *Proceedings of the 3rd International*

- symposium on the epidemiology and control of Salmonella in pork, Washington, DC. 39 – 42.
- International Organization for Standardization 6579 (ISO) (1998). Microbiology of food and animal feeding stuff-horizontal method for the detection of Salmonella, ISO, Geneva. 14-35.
- Mache A, Mengistu Y & Cowley S (1997). Salmonella serogroups identified from adult diarrhoeal out-patients in Addis Ababa, Ethiopia: Antibiotic resistance and plasmid profile analysis. *East Africa Medical Journal*, 74 (3): 183 – 186.
- Molla B, Alemayehu D & Salah W (2003). Sources and distribution of Salmonella serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. *Ethiopian Journal of Health Development*, 17, 63-70.
- Molla B & Mesfin A (2003). A survey of *Salmonella* contamination in chicken carcass and giblets in central Ethiopia. *Revue de Médecine Vétérinaire*, 154 (4): 267-270.
- Molla B, Mohamed A & Salah W (2006). Salmonella prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedaries*) in Eastern Ethiopia. *Tropical Animal Health and Production*, 36 (5):451-8.
- Moo D, O'Boyle D, Mathers W & Frost AJ (1980). The isolation of Salmonella from jejuna and cecal lymph nodes of slaughtered animals. *Australian Veterinary Journal*, 56: 181-183.
- Nabbut NH & Al-Nakhli HM (1982). Incidence of salmonellae in lymph nodes, spleens and of sheep and goats slaughtered in Riyadh public abattoir. *Journal of Food Protection*, 45: 1314-1317.
- NMSA National Meteorological Services Agency, Ethiopia (1996). Climatic and agro-climatic resources of Ethiopia. Addis Ababa. Internal Report. Meteorological Research Report Series, No.3, Addis Ababa, Ethiopia.
- Nyeleti C, Molla B, Hildebrandt G & Kleer J (2000). The prevalence and distribution of salmonellae in slaughter cattle, slaughterhouse personnel and minced beef in Addis Ababa (Ethiopia). *Bulletin of Animal Health and Production in Africa*, 48: 19-24.
- Quinn PJ, Carter ME, Markey BK & Carter GR (1994). Clinical Veterinary Microbiology. Wolfe Publishing, London.
- Samuel JL, Eccles JA & Francis J (1981). Salmonella in the intestinal tract and associated lymph nodes of sheep and cattle. *The Journal of Hygiene*, 87: 225-232.
- Wassie MA (2004). A cross-sectional study on Salmonella in apparently healthy slaughtered sheep and goats at Addis Ababa and Modjo abattoirs, Ethiopia.
- White DG, Zhao S, Sudler R, Ayers S, Friedman S & Chen S (2001). The isolation of antibiotic-resistant Salmonella from retail ground meats. *New England Journal of Medicine*, 345 (16): 1147-1154.
- Woldemariam E, Molla B, Alemayehu D, & Muckle A (2005). Prevalence and distribution of salmonella in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. 58:19-24.
- Wray C & Davies RH (2000). Salmonella Infections in Cattle. In: Wray C & Wray A. (Eds.). Salmonella in Domestic Animals. New York, CABI Publishing. 169-190.