Assessment of Risk Factors and Prevalence of *Salmonella* in Slaughtered Small Ruminants and Environment in an Export Abattoir, Modjo, Ethiopia

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Abstract: A survey study was conducted on 142 and 60 apparently healthy slaughtered sheep and goats, respectively, at an export abattoir, Modjo, Ethiopia from October 2007 to April 2008. The objectives were to determine prevalence of *Salmonella* in slaughtered sheep and goats and abattoir environment, investigate the potential risk factors and *Salmonella* contamination rates of carcasses. A total of 1,240 samples consisting of skin swabs, evisceration's hand swabs, eviscerating knife swabs, mesenteric lymph nodes, caecal contents, carcass swabs and water were collected. The samples were examined for the presence of *Salmonella* following standard techniques and procedures outlined by the International Organization for Standardization. From the total of 202 animals examined for *Salmonella,* 18 (8.9%) were positive, of which 11 (7.7%) were sheep and 7 (11.7%) were goats. In a total of 1,240 different samples, *Salmonella* was isolated in 89 (7.2%) samples of which 25 (12.4%) were carcass swabs, 11 (5.5%) mesenteric lymph nodes, 8 (4.0%) caecal contents, 10 (5.0%) skin swabs, 18 (8.9%) evisceration's hand swabs, 15 (7.4%) eviscerating knife swabs and 2 (7.1%) water samples. Salmonellae were detected in all test samples obtained from sheep and goats. No statistically significant results were observed except with the eviscerating knife swab, which was found to be significantly associated with carcass contamination. Sheep and goat carcasses that were eviscerated using *Salmonella* positive knives were 4.175 times more likely to be contaminated with *Salmonella* compared to those eviscerated with *Salmonella* negative knives.

Key words: Slaughtered sheep and goats · Salmonella · Prevalence · Modjo · Ethiopia

INTRODUCTION

Food safety has been a concern of mankind since the dawn of history. Despite advances in food science and technology, foodborne diseases remain one of the major public health problems all over the world; they are also an important cause of reduced economic productivity [1-3]. The world Declaration on Nutrition, adopted by the FAO/WHO International Conference on Nutrition, emphasizes that hundreds of millions of people suffer from communicable and non-communicable diseases caused by contaminated food and water [1, 3-4].

Food animals harbor a wide range of *Salmonella* serotypes and so act as sources of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis. The process of removing the gastrointestinal tract during slaughtering of food animals is regarded as one of the most important sources of carcass and organ contamination with *Salmonella* at abattoirs. Moreover, contamination of meat by

Salmonella may occur at abattoirs from the excretion of symptomless animals, contaminated abattoir equipment, floors and personnel and the pathogen can gain access to meat at any stage during butchering. Cross contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution [5- 7].

A number of studies conducted on poultry, pig, cattle, poultry meat, minced beef and humans in Ethiopia showed that salmonellae are prevalent in various food animals and meat products [6-11] and humans [12-13]. Although little study has so far been undertaken to determine the prevalence of *Salmonella* in sheep and goats in Ethiopia [7, 14] studies carried out elsewhere indicated that salmonellae are widespread in small ruminants [15-17]. Moreover, none of the previous studies in Ethiopia on small ruminants determined the occurrence, magnitude and distribution of *Salmonella* in the environment where sheep and goats are slaughtered. Therefore, this study was undertaken at a Modern export

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Abattoir in Modjo with the objectives of determining the occurrence and prevalence of *Salmonella* in slaughtered sheep and goats and investigating the major sources of carcass contamination in abattoirs.

MATERIALS AND METHODS

Study Design and Sampling: A survey study was undertaken on apparently healthy slaughtered sheep and goats, apparently healthy abattoir personnel and the abattoir environment at an export abattoir at Modjo, Ethiopia from October 2007 to April 2008. The variable of interest considered as an output variable at the slaughterhouse was carcass *Salmonella* status. The explanatory variables considered were *Salmonella* status of sheep and goats' skin, eviscerating knives, evisceration's hands, mesenteric lymph nodes, caecal contents and the water used to wash the carcass.

The sample size required for this study was determined depending on the expected prevalence of *Salmonella* and the desired absolute precision according to Thrusfield, 2005 [19]. Previous study on *Salmonella* in sheep and goats in Modjo Export Abattoir recorded a prevalence of 10.3% and 3.9%, respectively [14]. Therefore, 95% confidence interval, 5% precision and respective 10.3% and 3.9% expected prevalence of sheep and goats were used to estimate the sample size. Accordingly, the number of slaughter sheep and goats needed to demonstrate the prevalence of *Salmonella* was estimated at 142 and 60, respectively.

Sampling Procedure: Individual animals were systematically sampled depending on the number of animals slaughtered on each day. Samples were collected weekly and on each visit 7 animals and equal numbers of environmental samples were collected.

From each selected slaughtered sheep and goats; skin swabs, mesenteric lymph nodes, caecal contents and carcass swabs were collected in separate sterile containers. Samples were also collected from eviscerating knives, evisceration's hand and water used to wash the carcass. The samples were then transported on ice to the Addis Ababa University, Faculty of Veterinary Medicine Microbiology laboratory, Debre Zeit, Ethiopia, for processing and analysis upon arrival.

Isolation and Identification of *Salmonella:* Salmonella was isolated and identified according to the techniques outlined in the International Organization for Standardization [20-21]. The bacteriological media used in different stages of the isolation and identification of *Salmonella* were prepared according to the manufacturer's recommendations. All samples were processed separately.

Pre-Enrichment in non Selective Liquid Medium: All the samples were processed separately and then the processed samples in appropriate amount of BPW (1:9) were incubated for 18h ± 2h at 37°C ±1°C.

Enrichment in Selective Liquid Media: Rappaport-Vassiliadis with Soya (RVS) broth (Titan Biotech Ltd. Bhiwadi, India) and Muller-Kauffmann tetrathionate with novobiocin (MKTTn) broth (Oxoid Ltd. Basingstoke Hampshire, England) were used for selective enrichment of all the samples except the caecal contents [21]. In the case of caecal content samples modified semi-solid Rappaport-Vassiliadis (MSRV) medium (Bacto®, Difco Laboratories, USA) and MKTTn broths were used [20].

A 0.1 ml pre-enriched sample was transferred aseptically into a tube containing 10 ml of MSRV medium for caecal content samples or 10 ml of RVS broth for the remaining sample types and incubated at 41.5°C ±1°C for 24h ± 3h. Another 1 ml of the culture obtained in pre-enrichment broth was transferred aseptically into a tube containing 10 ml of MKTTn broth and incubated at 37°C ±1°C for 24h ± 3h.

Plating out and Identification: Xylose lysine desoxycholate (XLD) agar (Titan Biotech Ltd. Bhiwadi, India) and *Salmonella - Shigella* (SS) agar (Titan Biotech Ltd. Bhiwadi, India) plates were used for plating out and identification purpose.

A loopfull of inoculum from each RVS, MSRV and MKTTn broth cultures was streaked onto XLD and SS agar plates and the inoculated plates were incubated at 37°C for 24 ± 3h. After proper incubation, the plates were examined for the presence of suspected *Salmonella* colonies which were tested biochemically according to Mention author (s) [21].

Data Analysis: All data were transferred to SPSS release 11.5.0 for analyses. The agreement of the mesenteric lymph node and caecal content results was measured using the Kappa statistic. The data were analyzed by comparing proportions using Pearson's chi-square or Fisher's exact test based on the number of observations per contingency table cells. For association of risk factors considered in the abattoir with carcass contamination, multiple stepwise logistic regression analysis was used.

The explanatory variables considered (skin swab, eviscerating knife swab, evisceration's hand swab, caecal content, m esenteric lymph node and water sample *Salmonella* status and total slaughter volume) were separately analyzed to see their associations with the outcome of the bacteriological status of the carcass.

RESULTS

Prevalence of *Salmonella*: Out of the total 202 animals (142 sheep and 60 goats) examined for bacteriological status of *Salmonella*, 18 (8.9%) were positive of these, 11 (7.7%) were sheep and 7(11.7%) were goats. No statistically significant differences (p > 0.05) were found between sheep and goats in being positive for *Salmonella* (Table 1). An animal was considered *Salmonella* positive when it was bacteriologically positive either for MLN and/or CC. Skin and carcass *Salmonella* statuses were considered indicators of contamination and were not used for the calculation of prevalence. Of the 18 positive animals, only 1 (5.6%) animal was culture positive both for MLN and CC samples. The rest (94.6%) were culture positive either

for MLN or for CC samples and were not significantly different (p = 0.366). The agreement of the MLN and CC samples was measured using the Kappa statistics and the result indicated low agreement between the two (Kappa value = 0.062, 95% CI = -0.074 - 0.198).

Of the total 1,240 samples examined from sheep, goats, abattoir personnel and abattoir environment, *Salmonella* was isolated in 89 (7.2%) samples of which 25 (12.4%) carcass swab, 11 (5.5%) mesenteric lymph node, 8 (4.0%) caecal content, 10 (5.0%) skin swab, 18 (8.9%) evisceration's hand swab, 15 (7.4%) eviscerating knife swab and 2 (7.1%) water samples were positive for *Salmonella* (Table 2).

Salmonellae were detected in all test samples obtained from sheep and goats with different frequencies of occurrence. There were no statistically significant differences (p > 0.05) in the proportions of *Salmonella* between sheep and goat samples.

The level of carcass contamination was considered as an outcome variable taking skin swab, mesenteric lymph node, caecal content, evisceration's hand swab, eviscerating knife swab and water sample *Salmonella* status and total slaughter volume as risk factors for carcass contamination.

Table 1: Comparative results of species-specific *Salmonella* prevalence in MLN and CC samples at Modjo Export abattoir, Ethiopia,

Sample type	Odds Ratio	CI for the Odds Ratio	P-value
MLN	1.134	0.290 - 4.431	0.579
CC	0.237	0.055 - 1.027	0.052

MLN = Mesenteric lymph node, CC = caecal content, CI = confidence interval

Table 2: Prevalence of *Salmonella* by sample types and species of animals examined

	Number of samples								
	Sheep			Goats			Total		
Sample types	Examined	Positive (%)	95% CI	Examined	Positive (%)	95% CI	Examined	Positive (%)	95% CI
SkS	142	7 (4.9)	2.2 - 0.3	60	3 (5.0)	1.3 - 14.8	202	10 (5.0)	2.5 - 9.2
MLN	142	8 (5.6)	2.6 - 1.2	60	3 (5.0)	1.3 - 14.8	202	11 (5.5)	2.9 - 9.8
CC	142	3 (2.1)	0.6 - 6.5	60	4 (6.7)	3.1 - 19.1	202	8 (4.0)	1.9 - 7.9
CS	142	20 (14.1)	9.0 - 1.2	60	5 (8.3)	3.1 - 19.1	202	25 12.4)	8.3 - 17.9
HS*	142	15 (10.6)	6.2 - 7.1	60	3 (5.0)	1.3 - 14.8	202	18 (8.9)	5.5 - 13.9
KS*	142	12 (8.5)	4.6 - 4.6	60	3 (5.0)	1.3 - 14.8	202	15 (7.4)	4.4 - 12.2
WS*	20	1 (5.0)	0.3 - 6.9	8	1(12.5)	0.7 - 53.3	28	2 (7.1)	1.3 - 25.0
Overall	142	11(7.7%)	4.4 - 13.3	60	7 (11.7%)	5.8 - 22.2	202	18 (8.9%)	6.1 - 14.2

SkS = skin swab, MLN = mesenteric lymph node, CC = caecal content, CS = carcass swab, HS = evisceration's hand swab, KS = eviscerating knife swab,

WS = water sample, CI = confidence interval

* Sample types collected during sampling of the respective species of animals.

Table 3: Summary results of multiple stepwise logistic regression of the associations of carcass contamination with *Salmonella* with the risk factors

Risk factors	Coefficient	Std. Err.	P-value	Odds Ratio	95% CI for the Odds Ratio
SkS	0.608	0.821	0.459	1.837	0.367 - 9.186
MLN	1.058	0.714	0.138	2.881	0.711 - 11.673
CC	0.908	0.846	0.283	2.478	0.472 - 13.014
HS	0.796	0.613	0.193	2.218	0.668 - 7.367
KS*	1.429	0.597	0.017	4.175	1.297 - 13.444
WS	2.037	1.542	0.186	7.667	0.374 - 157.361
Total slaughter volume	0.000	0.001	0.576	1.000	0.999 - 1.001

Std. Err. = Standard error, CI = confidence interval, SkS = skin swab, MLN = mesenteric lymph node, CC = caecal content, HS = evisceration's hand swab, KS = eviscerating knife swab and WS = water samples, * significant difference

Therefore, associations of carcass contamination with the risk factors were assessed using logistic regression analysis (Table 3) and no statistically significant associations could be demonstrated between the carcass contamination and skin swab, mesenteric lymph node, caecal content, evisceration's hand swab, water sample *Salmonella* status and total slaughter volume ($p > 0.05$).

DISCUSSION

In the present study, the prevalence of *Salmonella* in apparently healthy slaughtered sheep and goats was 7.7 and 11.7%, respectively. These findings are in agreement with the report of D'Aoust, 1989 [22], which indicated that prevalence of *Salmonella* ranged between 2 and 51.5% in sheep and 1 - 18.8% in goats. Woldemariam *et al.* 2005 [7] reported respective prevalence of 2.8 and 9.8% in apparently healthy slaughtered sheep and goats, respectively in Debre Zeit, Ethiopia. In contrast, Wassie, 2004 [14] found higher prevalence of *Salmonella* in sheep of 11.5% as compared to goats of 3%. The current study revealed results, which are lower than the respective 14.7 and 18.3% prevalence in slaughtered sheep and goats in Riyadh Public Abattoir, Saudi Arabia [16] and the 17.6% prevalence of *Salmonella* in goats slaughtered for chevon in India [23]. The difference in the reported prevalence could be associated with the sampling plan and procedures, sample type, the 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 [24]. It is also known that keeping animals to be slaughtered in crowded waiting pens at abattoirs could facilitate the excretion and transmission of infection among them. In addition to this, stress could induce higher infection rates among animals when they are held in the market for long periods before slaughter [25-26].

The respective 2.1 and 8.3% *Salmonella* prevalence in caecal contents of sheep and goats obtained in this study compared well with the respective 2.1 % prevalence of *Salmonella* in sheep feces reported by Woldemariam *et al.* 2005 [7]. However, a study carried out to estimate the prevalence of fecal *Salmonella* in healthy pigs, cattle and sheep at slaughter in Great Britain yielded *Salmonella* prevalence of 0.1% in caecal contents of sheep [27], which is largely lower than the findings of the current study. It is well documented that, when animals are starved, salmonellae can survive and multiply in the rumen. Moreover, healthy carriers intermittently excrete only a few salmonellae, unless they undergo some kind of stress, for example during transportation or holding in the lairages prior to slaughter [16, 28-30]. Therefore, the present high caecal prevalence of *Salmonella* could be associated with the exposure of animals to such predisposing factors as starvation, overcrowding, transportation and longer lairage confinement prior to slaughtering.

The detection of *Salmonella* of 5.6 and 5.0% in the mesenteric lymph nodes of sheep and goats respectively, supports earlier observation by Moo *et al.* 1980 [28] who reported 4% *Salmonella* prevalence in mesenteric lymph nodes in Australian sheep. However, the current study findings considerably vary from other previous reports; Wassie, 2004 [14] reported 7.7 and 2% prevalence of *Salmonella* in the mesenteric lymph nodes of sheep and goats, respectively. Woldemariam *et al.* 2005 [7] also found respective 0 and 11.7% prevalence of *Salmonella* in sheep and goat mesenteric lymph nodes. According to Nabbut and Al-Nakhli, 1982 [6], the enrichment method revealed more infected lymph nodes than by direct plating method. Therefore, the differences in the reported prevalence could be associated with the bacteriological techniques employed for the detection of *Salmonella* or differences due to the distribution of the organisms in different study populations in different prevailing conditions. In the present study, there was no statistical

association between mesenteric lymph node *Salmonella* prevalence and carcass contamination. This could be due to the fact that lymph nodes are solid enclosed tissues so that they are not likely to contaminate hands of butchers, environment or carcasses, unless incised during inspection.

In this study, 7.1% (2/28) *Salmonella* prevalence was recorded in the water samples used to wash the carcasses. No comparable data is available for water used to wash sheep and goat carcasses. However, no *Salmonella* was recovered from 16 scalding water samples at Addis Ababa abattoir, Ethiopia [11] and in 5 Belgian slaughterhouses [31]. Moreover, a study undertaken to determine the level of selected bacteria in the water used for the rinsing of broiler carcasses at small retail processing operations in Trinidad resulted with 5.1% (4/78) *Salmonella* prevalence [32]\. The absence of *Salmonella* in scalding water seems to be due to high scalding water temperature recorded during slaughter activities. But, at this Modjo abattoir survey cold water was used to wash the carcasses of sheep and goats, which may have contributed to the relative high prevalence of *Salmonella* in it.

In the present study, 7.4% *Salmonella* prevalence from the eviscerating knives obtained in this study is nearly similar with the 5% prevalence of evisceration knife-study in Queensland, Australia [33] and the 5% prevalence on the killing knives in poultry slaughterhouses in Iraqi [34]. In contrast, another study on knife blades undertaken to indicate the prevalence of *Salmonella* reported 26.7 and 10% prevalence at two Botswana abattoirs, A and B, respectively [35].

However, eviscerating knife swab was found to be significantly associated (p = 0.017) with carcass contamination and the odds ratio (OR) was 4.175. Therefore, carcasses of animals that were eviscerated using *Salmonella* positive knives were 4.175 times (OR = 4.175, 95% CI = 1.297 - 13.444) more likely to be contaminated with *Salmonella* compared to those that were eviscerated using *Salmonella* negative knives. But, the biological significance of some potential risks showed high probability of associations for example, MLN with OR = 2.88, CC with OR = 2.48, HS with OR = 2.22 and WS with OR = 7.67.

A study was undertaken to examine salmonellae on posts, handrails and hands in a beef abattoir in Queensland. Salmonellae were isolated from the hands of workers in all stages along the slaughtering line with 30% on the hands of workers in the evisceration area [36].

Comparatively, that prevalence was higher than the 8.9% recorded in Modjo abattoir study. Watson, 1975 [25] described that washing of the hands with soap and running water for 15 seconds is needed to remove an inoculum of 100 or less of *Salmonella* organisms from the finger tips. But, heavier inocula leave viable salmonellae on the hands even a fter such washing. Similarly, Smeltzer *et al.* 1980 [36] indicated that washing is an essential part of any program aimed at reducing cross contamination of carcasses with *Salmonella*. Therefore, the low prevalence obtained in this study could be as a result of frequent hand washing which might have reduced bacterial loads from the hands of those personnel to low levels.

It is clear that the presence of *Salmonella* excretors in batches of animals in transit and passing through the lairage could result in contamination of skins. Moreover, Bacon *et al.* 2002 [37] indicated that external surfaces of animals serve as a source of contamination for the underlying, sterile carcass surfaces during the dehiding process. Examination of 100 cattle and 100 sheep passing through 10 abattoirs in Australia showed a high level of *Salmonella* contamination of the hides in cattle (57%) and fleece in sheep (51%) [25]. This finding is much higher than the findings of 4.9 and 5% *Salmonella* prevalence obtained from the skin of sheep and goats, respectively in this study. However, no statistically significant association was found between skin swab *Salmonella* prevalence and carcass contamination. The probable reason for this is that there was less contact between the skin and the underlying, sterile carcass surfaces as flaying, in the study abattoir, was carried out automatically. It has been indicated that manual operation of all the processing steps during slaughtering of the animals in abattoirs, rather than the use of semi-automatic or automatic systems in operations increases the probabilities of contamination of edible organs and spreading of salmonellae in abattoir environments [16].

This study recorded 14.1% prevalence of *Salmonella* on sheep carcasses and 8.3% on goats. These are in consistent with reports of previous works. Woldemariam *et al.* 2005 [7] reported respective 7.4 and 7.5% prevalence of *Salmonella* on carcasses of sheep and goats at Debre Zeit abattoir, Ethiopia and Sierra *et al.* 1995 [38] reported 10% prevalence of *Salmonella* on freshly dressed lamb carcasses in Spain. The high level of carcass contamination with *Salmonella* is of special public health significance for a country like Ethiopia, where raw and undercooked meat is the favorite meal in most areas.

In the present study, although the associations of carcass contamination with the potential risk factors was assessed, no statistically significant associations could be demonstrated between the carcass contamination and the risk factors except with eviscerating knife swab, which was found to be significantly associated with carcass contamination. However, there were other risk factors, which showed significant (OR > 1) associations with contamination of carcasses. Nevertheless, specific attention must be given to the sterilization of knives. As clearly indicated by different workers [25, 35, 39], it is salutary to note that knives must be immersed in water for 2 minutes at 82°C to reduce the number of contaminating microorganisms.

As indicated by Norval, 1961 [40], there is no doubt that the wiping cloths used by slaughter personnel for cleaning up the carcasses could be an important source of contamination of carcasses. In this survey it was observed that after washing the carcasses using pressurized water, slaughter personnel used wiping cloths to clean and dry the surface of the carcasses. Moreover, the wiping cloths used were not sterile and one wiping cloth was used for a number of continuous carcasses. This situation might considerably contribute to the cross contamination of carcasses resulting in relatively high prevalence of *Salmonella* on carcasses.

According to Smeltzer *et al.* 1980 [39], contact between aprons and the carcasses are unavoidable in many locations and may result in carcass-to-carcass transfer of *Salmonella*. In addition, results from different studies showed that equipment that indirectly or accidentally contacts the carcass such as steels, scabbards, aprons, protective rails, stainless steel sheets or other fixed structures does contribute to the spread of *Salmonella* in a meat work. All these factors may also contribute to the relatively higher prevalence of *Salmonella* on carcasses of the slaughtered sheep and goats obtained in this study. In conclusion, there was high contamination of sheep and goats carcasses with *Salmonella* indicating the role of slaughter processes followed by the abattoir in carcass contamination. Eviscerating knife was found to be the main source of carcass contamination during the slaughtering process. Therefore, good hygienic practices in the abattoir and applications of the hazard analysis critical control point concept should be put in place in order to eliminate or reduce foodborne pathogens to acceptable limits, including *Salmonella*.

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