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Risk Factors for *Salmonella* Contamination of Cattle Carcasses at the Jimma Municipal Abattoir, South West Ethiopia

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Abstract: *Salmonella* species are a leading cause of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in developing countries. The objective of this study was to determine the risk factors of *Salmonella* contamination in slaughtered cattle carcass at the Jimma municipality abattoir. A cross-sectional study was conducted among slaughtered cattle carcasses in the Jimma municipality abattoir from May to September 2016. A total of 195 cattle carcasses swabs were collected. To assess the risk factors, a fecal sample from each cattle was collected and observations of the hygienic condition of the procedure were documented using a checklist. The samples were examined for the prevalence of *Salmonella* following standard techniques and procedures outlined by the International Organization for Standardization. Out of the total 195carcasses samples *Salmonella* was isolated in 22(11.3%). Among factors studied fecal contamination was found to be significantly associated (P = 0.002) with *Salmonella* prevalence on the carcass. Finally the main sources of carcasses contamination were determined based on visuals observation of slaughter procedures and microbial examination. Severe limitations in hygienic practices were observed which require a serious attention from all relevant authorities to apply and maintain the basic hygienic slaughtering practices to prevent hazards which may affect the public health.

Key words: *Salmonella* · Risk Factors · Carcass · Cattle

INTRODUCTION

Food-borne diseases are public health problems both in developed and developing countries. An estimated 600 million almost 1 in 10 people in the world fall sick due to eating contaminated food and among these 420, 000 die every year. The main causes of foodborne illness are bacteria which constitutes 66% of the problems. Botulism, *Clostridium perfringens* gastroenteritis, *E. coli* infection, Salmonellosis and Staphylococcal food poisoning are the major food illness caused by bacteria [1, 2].

Salmonella species are a leading bacterial cause of acute gastroenteritis. Although the global human health impact of *Salmonella* infections has not been estimated, gastroenteritis is a major cause of morbidity and mortality, worldwide, both in children under 5 years old and in the general population [3, 4]. The Food Net active surveillance network estimated that non-typhoidal serovars cause 1.4 million human infections in the USA each year, resulting in 168 000 visits to physicians, 15 000 hospitalizations and 400 deaths [4] In the UK in 2007, 14060 laboratory-confirmed cases of non-typhoidal salmonellosis were reported [5] and also *Salmonella* has been reported to be the major pathogen causing food-borne illnesses in Africa, estimated to cause 33, 490 deaths in 2010 [6].

Cross-contamination of carcasses with *Salmonella* can occur during slaughtering operations at the abattoir. Stress associated with the transport of animals to abattoir augments the shedding of *Salmonella* by carrier animals and this causes spreading of the organism to other animals in the slaughter plant [7, 8]. During slaughter, fecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain [9, 10].

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In Ethiopia, several factors including unhygienic living circumstances and tradition of raw meat consumption may substantially contribute to the occurrence of Salmonellosis. Although surveillance and monitoring systems are not in place and its epidemiology is not described, qualitative and quantitative syntheses of previous studies could shed light on the occurrence of the disease and the major serotypes that frequently cause infections. Therefore, this study was aimed at determining the risk factors of *Salmonella* infection in the slaughtered cattle carcass at Jimma municipality abattoir.

MATERIALS AND METHODS

Study Area and Period: The study was conducted at the Jimma municipal abattoir from May to September 2016. There was no clear division of the slaughtering process into stunning, bleeding, skinning, evisceration, chilling, cutting, or frozen delivery in the Jimma municipality abattoir. Bleeding and evisceration were conducted on a horizontal position on the floor by incising the hide at the bottom of the abdomen without flying the skin. Workers hoisted the carcass manually using a chained pulley system after flying the skin and evisceration on the floor. There were no knife and ax sharpening machines. There were no means of sterilizing equipment. Carcasses were manually quartered using axes.

Study Design and Population: A cross-sectional study was conducted to find out the risk factors of *Salmonella* infection of slaughtered cattle carcass at the Jimma municipality abattoir. The study population was all apparently healthy cattle slaughtered in the slaughterhouse.

Observation Checklist:

- A man who is responsible for slaughtering activity has washed his hands with soap or with other detergent material before slaughtering and after removal of the intestinal organs. Yes No
- A man who is responsible for slaughtering activity is wearing clean garment or gown during the procedure. Yes No
- How is the hygienic condition of the knife and ax? Good Bad
- Is carcass washed after slaughtering process with pipe water? Yes No
- Is slaughter floor cleaned by using pipe water and detergent material before and after slaughtering process? Yes No
- Note: For each variable in the study, cleanliness was evaluated using the following criteria:
- Clean ~ no visible fecal, blood and other dirty material on the surface of the equipment.
- Dirty ~ the surface of the equipment was covered with fecal, blood and other dirty material.

Sample Collection, Isolation and Identification Procedure: Study samples were selected using simple random sampling technique and 195 slaughter cattle were sampled. Observation checklist was used to document about the process. Sample collection, isolation and identification were made based on the recommendations of the International Organization for Standardization (ISO), 6579:2002 [11].

One hundred and nightly five (195) carcass swabs were collected. Each carcass was sampled on four regions, i.e., from the neck, brisket, flank and rump region. The area sampled in each region was 100 cm^2, resulting in a total area of 400 cm^2, using different pre-moistened commercial beef carcass sampling poly wipe kits and the swabs were transferred to a sterile plastic cup containing 10 ml of buffered peptone water. In addition, one (1) gram of feces from the rectum of the cattle was collected and transferred into to 9ml of buffered peptone.

Homogenized carcass and fecal sample were incubated at 37°C. Then, 1ml and a 0.1ml aliquot of the enrichment broths was transferred aseptically into 10 ml of Selenite Cystine and 10ml of Rappaport–Vassiliadis with soy broth and incubated for 24 hours at 37°C and 42°C, respectively. Following incubation, a loop full of each culture was streaked onto Brilliant Green Agar and Xylose Lysine Deoxycholate agar plates and incubated at 37°C for 24 to 48 hours. The plates (BGA and XLD) were examined for the presence of characteristics associated with *Salmonella* colonies. A single positive colony showing red color with a black center on XLD and red color on BGA agars were subjected for biochemical tests for confirmation.

Biochemical Tests: *Salmonella* isolates were identified using triple sugar iron agar, lysine iron agar, urea broth, indole test and citrate utilization tests. These were incubated for 24 to 48 hours at 37°C. Colonies producing an alkaline slant with the acid bottom and hydrogen

sulfide production on TSI, positive for lysine, negative for urea hydrolysis, negative for indole test and positive for citrate utilization were considered as *Salmonella* [9].

Data Quality Assurance and Analysis: All the instruments used for sample processing were checked prior to the study. Proper functioning was checked using quality control strains of *Salmonella Typhimurium* (ATCC 14028) and *E. coli* (ATCC 25922). Data consistency and completeness were made all the way during data collection, data entry and analysis. Data were edited, cleaned and checked for its completeness and entered into Epi Data 3.1 then exported to Statistical packages for social sciences (SPSS) Version 20 for analysis. Bivariate analysis was performed for each variable to select variables candidates for Multivariate analysis. Variables in bivariate analysis with a p-value <0.25 were taken as candidates for Multivariate analysis. P-value< 0.05 in the Multivariate analysis was considered as statistically significant.

RESULTS

A total of 390 samples, 195 carcass swabs and 195 cattle feces were collected from the Jimma municipality abattoir. From 390 samples, 31(7.9%) were positive for *Salmonella*. Of these, 22(11.3%) were detected from carcass swabs and 11(5.6%) were detected from fecal samples of animal (Table 2).

In the current study from 195 sample population, only 72(36.9%) of cattle were slaughtered with a washed knife. Our study showed that 159 (81.5%) of cattle were slaughtered with slaughter men without wearing the clean garment. Out of the total sample, 64 (32.8%) cattle were slaughtered on slaughter floor which was not cleaned. It was also observed that the slaughter men did not wash their hands in 139(71.3%) of the slaughtering procedure. Our finding showed that 116 (59.5%) of the carcasses were not washed with water during slaughtering and after finishing the slaughtering processes. In the Bivariate analysis of the variables, only the fecal material was found significantly associated with *Salmonella* infestation of the carcass (Table 1).

Table 1: Bivariate logistic regression analysis of variables considered in the analysis of risk factors for isolation of *Salmonella* in carcass at the Jimma municipal abattoir from May to

		Isolation of *Salmonella* in carcass			
Risk factors	Parameter	Positive (%)	Negative (%)	COR (95% CI)	p-value
Fecal material	Positive	5(45.5)	6(54.5)	8.19(2.26-29.66)	.001
	Negative	17(9.2)	167(90.8)		
Hand washing	Yes	8(14.3)	48(85.7)	1.49(.59-3.77)	.402
	No	14(10.1)	125(89.9)		
Using washed knife and ax	Yes	5(6.9)	67(93.1)	.47(.16-1.32)	.151
	No	17(13.8)	106(86.2)		
Cleaned slaughter floor	Yes	5(7.8)	59(92.2)	.57(.20-1.62)	.289
	No	17(13)	114(87)		
Using clean garment	Yes	15(11.6)	144(88.4)	.43(.16-1.15)	.093
	No	7(19.4)	29(80.6)		
Carcass washed	Yes	8(10.1)	71(89.9)	.82(.32-2.06)	.674
	No	14(12.1)	102(87.9)		

September 2016

Table 2: Multivariable logistic regression analysis of variables considered in the analysis of risk factors for isolation of *Salmonella* in carcass at the Jimma municipal abattoir from May to September 2016

		Isolation of *Salmonella* in carcass			
Risk factors	Parameter	Positive (%)	Negative (%)	AOR (95%CI)	p-value
Fecal material	Positive	5(45.5)	6(54.5)	7.76(2.09-28.86)	.002*
	Negative	17(9.2)	167(90.8)		
Using washed knife and ax	Yes	5(6.9)	67(93.1)	.58(.19-1.80)	.345
	No	17(13.8)	106(86.2)		
Using clean garment	Yes	15(11.6)	144(88.4)	.54(.18-1.61)	.270
	No	7(19.4)	29(80.6)		

* Statistically significant association

Fecal material, cleanliness of knives and ax and wearing clean garment were included into the final multivariable logistic regression and it showed that fecal material was statistically significantly associated (P<0.002) with *Salmonella* contamination of the carcass (Table 2).

DISCUSSION

In this study, from the total of 195 cattle carcass examined, 22 (11.3%) were positive for *Salmonella*. This is particularly important in Ethiopia where raw and undercooked meat is consumed. Our finding is in line with a study conducted in Addis Ababa, Ethiopia, 14.7% [12] and 11.8% from Selangor, Malaysia [13]. The prevalence reported in the current study is higher than other reports such as USA 1% [14] Canada 0.1% [15] Australia 0.22% [16] and 3.3% at Brazilian slaughterhouse [17]. This variation might be due to poor feeding practices, vaccination status of the animal, slaughterhouse infrastructure, variation on slaughtering equipment, slaughtering procedures, personal hygiene and personal education status of the personnel.

In the present study, the proportion of *Salmonella* isolated from a fecal specimen of slaughtered cattle was 5.6%. This is in agreement with studies conducted in Ethiopia 5.9% [18] and 6% [19]. However, the proportion we found is higher as compared to study conducted in Japan 0.5% [20] Great Britain1.4% [21] and 2% at Irish commercial abattoir [22]. The high prevalence of *Salmonella* detected in our study might be explained partly by the method used for strain isolation and partly by the animal husbandry practices. In Ethiopia, cattle mostly roam freely at pasture in the bush. The wild animals, such as hedgehogs, living in such places could contaminate grass with their excreta, which might contribute to the high prevalence of *Salmonella* in the current study. The current finding also revealed that fecal material was found to be significantly associated with *Salmonella* contamination of carcass. This result is similar to that reported in a previous publication in the USA [23], Mexico [24] and Ethiopia [19] which suggested that there is a strong correlation between the number of live animals that carry *Salmonella* spp. in their feces and the number of contaminated carcasses at the end of the slaughter line.

Hand hygiene is not a new concept for prevention of microbial contamination of food in the food industry. Unfortunately, hand hygiene is neither always carried out nor effectively implemented [25]. In the present study majority of the slaughtering operators in Jimma municipality abattoir did not wash their hands before slaughtering and after intestinal removal of the cattle. Studies conducted in Tanzania and the United Kingdom, also reported that 37.5 and 29% of abattoir workers, respectively, did not wash their hands before handling meat [26, 27]. In an extensive review on hand washing, the reasons for food handling personnel not washing their hands at appropriate times are laziness, time pressure, inadequate hand washing facilities and supplies, lack of accountability and lack of involvement by industry management and workers in supporting proper hand washing [28].

A study conducted in the USA reported that food handlers can be vectors for cross-contamination of food whenever good personal hygiene or proper food handling practices are not realized. Meat handler clothing can be a possible source of bacteria which can be transferred to meat during handling [29]. Our finding showed that 64 (32.8%) of operators did not wear clean garment during slaughtering activity. Similarly, a study conducted in Mekele, Ethiopia and Kampala, Uganda reported that 11.3 and 31.5% of abattoir workers, respectively, did not use clean garment during preparing meat [30, 31]. As reported by other studies the low usage of clean protective clothing in the study sites is indicative of increased risk of contamination of meat by abattoir workers [32, 33].

The study shows that cleaning and sanitizing of the slaughterhouse were a key component of good practices at a slaughterhouse and can confer significant benefits in terms of reducing the incidence of *Salmonella* contamination to the carcass [34]. The current study revealed that 64(32.8%) of cattle were slaughtered on an unclean slaughter floor. A similar study conducted in Bahir Dar, Ethiopia reported that 10 (10%) of cattle were slaughtered on the unclean floor [35].

The occurrence of *Salmonella* on the external surfaces of cattle carried into a slaughterhouse can serve as an indication of contamination that could potentially be transferred to carcass surfaces during the dehiding process. It is also subjected to contamination by the digestive flora during eviscerating. So, carcass washing plays a great role in reducing the prevalence of *Salmonella* at the slaughterhouse [36]. However, in the current study 116 (59.5%) of cattle carcasses were not washed by water.

A study conducted in Ethiopia showed that the eviscerating knife was found to be significantly associated with carcass contamination by *Salmonella* [37]. The similar finding reported in India also showed that cutting equipment are the major contaminants of fresh

meat in the slaughter plant [38]. Our observation during the survey showed that 123(86.2%) of cattle were slaughtered by using an unclean knife in Jimma municipality abattoir.

CONCLUSIONS

Results of the present study indicated that *Salmonella* is widespread in Cattle carcass. The study revealed that majority of Jimma municipal abattoir personnel did not adhere to the required sanitation and hygiene standards. All the sanitation and hygiene handling practices investigated could provide ways for contamination of meat and possibility of occurrence of *Salmonella*. The fecal material proved to be significantly associated with the prevalence of *Salmonella* in carcasses. Therefore particular attention should be paid to the herd contamination levels of incoming animals and the post-evisceration environment to better control *Salmonella* in cattle at the slaughterhouse. Proper cooking of meat before consumption and improving personal and meat hygiene in the line of meat production from farm to fork should be adopted to ensure the safety of meat and meat products for human consumption.

Declaration

Ethics Approval and Consent to Participate: The study was conducted after obtaining ethical clearance from Jimma University Health Institute, Faculty of Health Sciences and Ethical Review Board. Permission letter was obtained from Jimma town municipality Office and submitted to the Abattoir administration.

Consent for Publication

Not Applicable

Availability of Data and Materials: The data sets developed and/or analyzed during the current study are available from the first author or from the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no competing interests.

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Abbreviations:

BGA: Brilliant Green Agar and, TSI: triple Sugar Iron agar,

XLD: Xylose Lysine Deoxycholate agar.

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