

Prevalence of Salmonella in Butcher Houses in Jimma Town, Ethiopia

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Abstract: The present study was undertaken to determine the prevalence of salmonella in meat in butcher houses located in Jimma town during the period of November 2014- 2015. A total of 25 butcher houses were purposely selected and examined for presence or absence of salmonella. Three different samples, abdominal muscle, Liver and Mesenteric lymph node were collected during each sampling time. A total of 409 samples were tested and out of which salmonella was isolated from 161 (39.4%) of the total samples. This comprised of 56(13.7%) from the Abdominal muscle, 46(11.2%) from liver and 59(14.4%) from Mesentric lymph node. We have seen the prevalence of salmonella based on different risk factors. Materials hygiene was 125(58.7%), worker hygiene were 76(42.9%) and hotels hygiene 152(96.2%) were identified.

Key words: Butcher Houses • Ethiopia • Jimma town • Prevalence • Salmonella

INTRODUCTION

The prevalence of Salmonella has been widely reported in cattle [1]. Salmonella still represents a major public health problem in Ethiopia especially in most parts of country and infected animals may shed the organism in their faces without showing any clinical signs of disease [2].

In humans, Salmonella is one of the most common causes of bacterial gastroenteritis [3]. About 20% of human salmonellosis is associated with contaminated pork products [4, 5]. Salmonella infection has been associated with many different food types and the consumption of beef has been associated with a number of outbreaks [6]. Salmonella is still one of the major global causes of gastroenteritis in humans and animals [2].

Salmonella has been detected in several locations within dairy farms and slaughterhouses, both before and after sacrifice; the same Salmonella clone has been recovered in a dairy herd and in ground meat products following processing. Consequently, the presence of Salmonella in cattle at slaughter and the consequent cross-contamination of edible carcass tissue presents a significant food safety hazard [7]. Salmonella is the leading cause of common food-borne infection in many

countries in the world. It is more widespread in young children, elderly citizens frequently affected with underlying chronic diseases and immuno-suppressed individuals in a study made by Bangui [8].

Salmonella *enteritidis* (*S. enteritidis*) or Salmonella *typhimurium* (*S. typhimurium*) most often cause acute diarrhea in individuals with AIDS and have been shown to be at higher risk than the general population for Salmonella spp infections. Human salmonellosis from the consumption of contaminated foods generally remains on the increase worldwide causing pain, suffering and loss of leisure time [9-11].

There are over 2500 different Salmonella serotypes and all are considered pathogenic to humans [12]. However, relatively few serotypes are associated with cattle and of these, Salmonella *enteric* subsp. *enterica* serotype Dublin (*S. Dublin*) and *S. enterica* subsp. *enterica* serotype *typhimurium* (*S. typhimurium*) are the most common in the UK and Ireland [9-11]. The presence of *S. typhimurium* in cattle and the consequent cross contamination of beef carcass tissue is of particular concern as this serotype is one of the most common causes of Salmonella infection in developed countries [13]. In addition to causing infection, many *S. typhimurium* isolates (although not exclusively isolates

of this serotype) have developed resistance to multiple antibiotics. Of particular note is *S. typhimurium* definitive type (DT) 104. Many isolates of this phage type are resistant to ampicillin chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT), with an increasing number of isolates showing resistance to *trimethoprim* and fluoroquinolones [14, 15].

Given the association of certain bovine *Salmonella* serotypes with food poisoning and the likelihood that some isolates may be multiplied resistant to antibiotics, a complete understanding of the risk posed by this pathogen during beef processing requires that the serotype and antibiotic resistance profile of isolates be determined in addition to the prevalence [16].

Despite the presence of a number of published information on different food items, little information is available on the status of *Salmonella* in apparently healthy slaughtered cattle in commercial abattoirs in Ethiopia [16]. Therefore this study was undertaken with the following objectives:

- To establish the prevalence of *Salmonella* in butcher houses.
- To determine the level of meat contamination.
- To determine sources of carcass contamination and institute some recommendations on how to avoid such contamination and to identify risk factors associated with the detection of *Salmonella* in butcher houses.

MATERIALS AND METHODS

Study Area: The study was conducted in Jimma town by taking samples from butcher houses. Jimma town is located 352km south-western of Addis Ababa at altitude of about 7013'-8°56'N and longitude of about 35052'-37037'E and at an elevation of 1750m above sea level and receives a mean annual rainfall of about 1530mm which comes from the long and short rain seasons. The annual mean minimum and maximum temperature 14.4 and 26.7 degree Celsius respectively [17].

Study Period and Design: A cross-sectional experimental study was conducted from November 2014 to April 2015 on ready-to- eat meat collected from butcher houses in order to assess the level of salmonella contamination of the meat after being collected from the slaughterhouse and assess the risk factors of meat contamination in butcher houses.

Study Methodology

Sampling Methodology: All samples were collected from different butcher houses which located in Jimma town. Mesenteric lymph nodes, liver and abdominal muscle samples were collected from butcher houses. The transportation method wear a hand glove to cut meat and put it in container, covered with it a cover then put it in ice box. The butcher houses from which I was going to collect the relevant samples are randomly selected using the identification number given to sample during sample collection. Butcher houses samples are collected weekly on Saturday. The mesenteric lymph node, abdominal muscle and liver were collected in separate sterile sample containers. Samples were also collected from different part of meat like mesenteric lymph nodes, abdominal muscle and liver. Samples from butcher houses were collected from 25 butcher houses per week.

About 25 gm. mesenteric lymph node samples have been collected aseptically, the same amount of abdominal muscle and liver content samples have been collected in sterile universal bottles aseptically. The entire outer surface of each carcass (both sides) will be rubbed over once from the hindquarter to the forequarter, uniformly using a fresh sterile sponge/cotton [16].

Isolation and identification of *Salmonella*: *Salmonella* was identified and isolated according to the techniques recommended by the International Organization for Standardization The detection of *Salmonella* necessitates different successive stages. The bacteriological media used for Isolation and identification was prepared according to the manufacturer's recommendations ISO 6579 [18].

Pre-enrichment: Twenty five gram of mesenteric lymph nodes was weighed and cut into smaller fine pieces with sterile scalpel blades on sterile plates and put in a sterile stomacher bag. Buffered peptone water (225ml) was added to the minced lymph node and homogenized using a stomacher bags. Fifteen ml of BPW was added to carcass swab samples and are incubated while kept in their original plastic bags. The pre-enrichment samples were incubated for 16 to 20 hours at 37°C.

Selective Enrichment: Selenite cysteine (SC) broth media was used for selective enrichment. After incubation, 1 ml of pre enrichment broth was transferred aseptically into

10ml of selenite cysteine and incubated at 37°C for 18 to 24 hours.

Selective Plating and Identification: Brilliant green-phenol red-lactose-sucrose (BPLS) agar and xylose lysine desoxycholate (XLD) agar plates will be used for this purpose. A loop full of inoculum from each Rappaport-Vassilliadis and selenite cysteine broth cultures will be streaked onto BPLS agar plates and XLD agar plates. The inoculated plates will be incubated at 37°C for 24 to 48 hours. After incubation, the plates will be examined for the presence of Salmonella colonies. Typical colonies of Salmonella grown on BPLS agar give an alkaline reaction and have red colonies while on XLD medium they produce hydrogen sulphide and have red colonies with a black (H₂S) center ISO 6579 [18].

Confirmation: For confirmation, at least five presumptive (typical or suspect) Salmonella colonies will be selected from every selective plating media. If the suspected colonies on each plate are fewer than five, all the colonies will be selected. The selected colonies will be streaked onto the surface of nutrient agar plates, in a manner which will allow well-isolated colonies to develop. The inoculated plates will be incubated at 37°C for 18 to 24 hours. The pure cultures on nutrient agar will be used for biochemical test ISO 6579 [18].

Biochemical Tests: The majorities of salmonellae are non-lactose fermenters and produce pale colonies on MacConkey agar and an alkaline reaction in the medium. However, it must be remembered that some strains of Salmonella arizonae are lactose positive and strains of *S. Typhimurium* have been encountered carrying plasmids with genes coding for lactose fermentation. Most salmonellae give an alkaline reaction in brilliant green agar and have red colonies. On XLD medium the majority of Salmonella serotypes produce hydrogen sulphide and have red colonies with a black (H₂S) centre. Colonies characteristic for Salmonella on the selective/indicator media are inoculated, singly into a triple sugar iron (TSI) agar slope and lysine decarboxylase broth. The typical reaction for Salmonella in TSI agar is a red (alkaline) slant, yellow (acid) butt and superimposed (black) H₂S production (R/Y/ H₂S+). The test for lysine decarboxylation is positive. However, *S. Choleraesuis* does not produce H₂S although *S. Choleraesuis* biotype kuzendorf is H₂S positive. Salmonellae are oxidase

negative and catalase positive, grow on citrate as the sole carbon source, do not hydrolyze urea and produce acid and gas from glucose in TSI. If the reaction in TSI and lysine decarboxylase broth is equivocal, further biochemical tests should be carried out or an identification system used such as API 20E (Analytic products) [18]. Salmonella generally are α -galactosidase, Voges-Proskauer and indole negative ISO 6579 [18].

The prevalence of Salmonella species as a biochemically homogeneous group of microorganisms is rapidly diminishing. The situation will likely lead to a reassessment of the diagnostic value of biochemical traits and to their likely replacement with molecular technologies targeted at the identification of stable genetic loci and/or their products that are unique to the genus Salmonella [19].

RESULTS

During sample collection, observation of the operations of the study butcheries revealed that study butcheries had different operating conditions and hygiene practice. All the butcheries displayed meat (beef) mixed with offal's openly on tables and wooden logs, had no screens which let flies into the butcheries, floors were not clean, knives and other into the butcheries, floors were not clean, knives and other Cutting tools were handled carelessly, weighing scales were unclean and all the butcheries lacked hand washing facilities.

A total of 409 samples were examined from 25 different butcher houses located in Jimma town. The prevalence of salmonella from a total sample during the study period was 161/409. Out of 161 salmonella-positive samples 56(13.7%) were from the abdominal muscle, 46(11.2%) from the liver and 59(14.4%) were from the mesenteric lymph nodes.

Level of salmonella contamination by the sample collected from different butcher houses located in Jimma town. MLN=Mesenteric lymph node; ABM; Abdominal muscle

This study also found out that the level of salmonella contamination in butcher houses in Jimma town was 39.4% (161/409).

Proportion from each positive sample from total positive are abdominal muscle 34.8%, liver 28.6% and mesenteric lymph nodes 36.65% were explained according to the Table 1.

Table 1: Prevalence of salmonella in butcher houses in different organs

Sample type	Infected	Prevalence (%)	Proportion for individual all organ to all sample size (%)	χ^2 (P-value)
Abdominal muscle	56	13.7	38.6	6.356(.047)
Liver	46	11.25	31.8	
Mesenteric lymph node	59	14.4	29.6	

Table 2: Prevalence of salmonella in butcher houses in different organs

Sample type	Number Examined	Positive	χ^2 (P-value)
Abdominal muscle	158	56(13.7)	6.356(.047)
Liver	130	46(11.2)	
Mesenteric lymph node	121	59(14.4)	
Total			

Table 3: Materials Hygiene relation to salmonella infected organs

Risk factor	Positive	Total	χ^2 (P-value)
Poor	125(58.7%)	213	69.515(0.000)
Good	36(18.4%)	196	
Total			

Table 4: Worker hygiene relation to salmonella infected organs

	Negative	Positive	Total	χ^2 (P-value)
Poor	101	76	177	1.669(1.96)
%within worker hygiene	57.1%	42.9%	100%	
Good	147	85	232	
%within worker hygiene	63.4%	36.6%	100%	

Table 5: Hotels hygiene relation to salmonella infected organs

	Negative	Positive	Total	χ^2 (P-value)
Poor	6	152	158	
% within hotels hygiene	3.8%	96.2%	100%	
Good	242	9	251	
%within hotels hygiene	96.4%	3.6%	100%	

DISCUSSION

The prevalence of salmonella infection in this study was 39.4% (161). However, there have many studies on salmonella infection in butcher houses, there has no uniformity with respect to hotel hygiene, workers' hygiene and materials hygiene and sampling cultural techniques, consequently, the result may not be comparable to Wray and Davies [12].

The present study demonstrated a high prevalence of salmonella infection in butcher houses in different organs which under took examined in Jimma town (39.4%).

In this study prevalence of salmonella in mesenteric lymph nodes and abdominal muscle are 59(48.8%) and 56 (35.4%) when we compare the results of this study with in other studied on mesenteric lymph nodes and abdominal muscle, it has been found that there were high prevalence of salmonella in our study and it was low in others because of different site of sample took and different risk factors.

The present finding showed a high proportion of infected mesenteric lymph nodes and abdominal muscle when compared with other studies conducted in Ethiopia by Nyeleti *et al.* [20] (2.2%) and (9.8%) in mesenteric lymph nodes and abdominal muscle respectively. In addition, Alemayehu *et al.*[21] reported 4.5% *Salmonella* contamination in the mesenteric lymph node samples of slaughtered cattle in Ethiopia.

However, compared to other countries, Salmonella contamination rates in mesenteric lymph node samples in this study prevalence in mesenteric lymph node was more related in Australia where, Frost *et al.* [22] (46.67%) and lower than Samuel *et al.*[23] (54%) reported respectively and again lower than, Moo *et al.* [24] reported a contamination rate as high as 71.76%. On the other hand, in Senegal *Salmonella* contamination in beef samples at slaughterhouses was 43%, Stevens *et al.* [25]. which was more related with this study.

Furthermore, in Thailand, *Salmonella* prevalence in beef samples was in the range 22.3-66.7%, Angkitittrakul *et al.* [26]. The prevalence of salmonella in related to materials hygiene in this study was very high which 125 were (58.7%). When compare this study with other which conducted by Legesse *et al.* [27], (5.6%). In our study prevalence of salmonella related to materials hygiene was very high.

The prevalence of salmonella related to workers' hygiene in this study was 42.9% which is lower when compared with Endale and Hailay [28] reported that 91.7% of the butcher houses workers in Mekelle city handle money while processing the meat. In addition, another study indicates that handling foods with bare hands may also result in cross-contamination, hence the introduction of microbes in to safe food. The hygienic practices at the butcheries are unhygienic. Almost all butcher house workers (42.9%) handle money with bare hands while processing meat and do not put on appropriate protective clothing. Because meat handlers are probable sources of contamination for microorganisms, especially salmonella. It is important that all possible measures to be taken to reduce or eliminate such contamination [29]. The existence of Salmonella in meat indicates that the contamination is of human origin and the result of poor personal hygiene during the handling and processing of food. The overall

butcher houses worker practices are favourable for the contamination of bovine meat with salmonella. In addition, most butchers wash their hands after the selling process and use only water with no detergents and use a single knife for edible offals and meat types and a single cutting board for all products without cleaning and sterilizing.

Prevalence of salmonella In related to hotels hygiene in this study was 152(96.2%) which is high when compare with Tegegne and Ashenafi [30] reported *Salmonella* contamination rate of 42% from minced meat (locally known as «kitfo») samples collected from different butcher houses, hotels, bars and restaurants in Addis Ababa.

CONCLUSION AND RECOMMENDATIONS

The present study results revealed the high prevalence of Salmonella, the presence of poor personal hygiene, hotel sanitation and materials hygiene, the low level of public awareness about the contamination of bovine meat with Salmonella and the associated probable risk in the environment. Most of the butcher houses were not in good status in case of site nears to car road and for contamination of meat by much dust from road by air etc.

Based on the above conclusion the following points are recommended:

- Training programs must be provided on best practices of handling of meat for handlers and raising the level of awareness of people.
- It is better if other studies regarding bacterial load and sources of contamination in the abattoir and butchers shops were performed.
- The further study ought to be conducted to identify the source of contamination.
- The government has to regulate the site of butcher houses.
- Environmental workers have to do strictly on many butcher houses.
- Trade and industry agencies before giving worker permission have put a guide which butcher houses have.

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