Salmonella spp. and risk factors for the contamination of slaughtered cattle carcass from a slaughterhouse of Bahir Dar Town, Ethiopia

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PEER REVIEW

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Comments
Studies of this kind are especially important in countries like Ethiopia where hygienic practices fall below the expected level, and consequently the prevalence of bacterial infections from consumption of raw and half−cooked meat is considerably high and will be a risk factor from the point of view of personal and public health. The authors have carefully chosen their investigation and executed it successfully. The methodology followed meets the standards expected, and the results obtained are apparently reliable. The discussions made on the results are sound, and the conclusions drawn are appropriate. The corrective measures suggested by the authors should find general acceptance. The literature survey is thorough, with quite a few recent and appropriate references included.

ABSTRACT

Objective: To assess the prevalence of *Salmonella* species and associated factors from cattle carcasses in abattoir of Bahir Dar, Ethiopia.
Methods: A cross−sectional study was conducted among slaughtered cattle carcass samples from Bahir Dar abattoir from December 2012 to February 2013. A total of 300 carcasses from hind limb, abdomen and neck of one hundred cattle were collected and analyzed for the prevalence of *Salmonella*. An observation checklist was used to collect data on the risk factors for contamination of carcass.
Results: Out of the total 300 carcass samples, 23 (7.6%) were positive for *Salmonella* species. Eleven (47.8%), nine (39.1%) and three (13.1%) of the isolates were *Salmonella* group A, *Salmonella arizonae* and *Salmonella typhi*, respectively. Wearing garment during slaughtering, hand−washing after separating intestinal content, washing of the knife before slaughtering, slaughtering on sanitized floor and carcass−washing during slaughter were important risk factors that have statistically significant association with the isolation rate of *Salmonella* in slaughtered cattle carcasses ($P<0.05$). The mean aerobic mesophilic count of cattle carcass surfaces was 5.39 log CFU/cm^2.
Conclusions: Unlike other studies, *Salmonella* group A appears to be the most prevalent species in cattle carcasses in the present study. Among carcass sites, the highest contamination was recorded in the abdomen and the main source of contamination is human and intestinal contents. Therefore, there is a need to maintain proper hygienic practices in Bahir Dar abattoir during slaughtering.

1. Introduction

Salmonella species are a leading cause of acute gastroenteritis in several countries, and salmonellosis remains an important public health problem worldwide, particularly in the developing countries[1]. The presence of *Salmonella* in food animals at slaughter and the consequent cross−contamination of edible carcass tissues present

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a significant food–safety hazard[2]. Food animals such as cattle may carry *Salmonella* at slaughter and can serve as sources of contamination and provide an opportunity for entry of the pathogen into the food products. This implies that the presence of *Salmonella* in slaughter cattle and slaughterhouse environment, and the potential cross–contamination of carcasses and edible organs can pose food–safety hazards[3].

Raw meat coming from slaughterhouse is available in open–air local retail shops without appropriate temperature control, and this is purchased by households and also partially cooked minced meat is served in restaurants. Although there are a few studies done on the prevalence and antimicrobial susceptibility of *Salmonella* serotypes on apparently healthy slaughtered cattle, no study has been done on the microbial load and risk factors of contamination during slaughtering. A periodic surveillance on the prevalence of *Salmonella* and risk factors in slaughtered food animals is essential to control the spread of the pathogen and infection in man through contaminated carcass[4]. This is required to suggest the acceptance of the carcass in relation to the standards. The objective of this study was therefore to investigate the prevalence of *Salmonella* and associated factors from slaughtered cattle in abattoir of Bahir Dar, Ethiopia.

2. Materials and methods

2.1. Study design

A cross–sectional study was conducted from December 2012 to February 2013 to assess the isolation rate of *Salmonella* species in cattle carcass samples and associated factors from abattoir of Bahir Dar, Ethiopia.

2.2. Sampling

A total of 300 carcass (one hundred from each of hind limb, abdomen and neck) samples were collected from 100 randomly selected cows. About 100 cm^2 of cattle carcass surface around the hind limb (medial), abdomen (lateral) and neck was swabbed by wiping the cotton swabs on each sampling site, five times in both vertical and horizontal directions for 30 seconds using sterile surgical glove[2]. The swab samples were transported from the site of collection to the Bahir Dar University Postgraduate Microbiology Laboratory using an icebox within one hour of collection. The swab samples were analyzed immediately for isolation of *Salmonella* and aerobic mesophilic counts.

2.3. Culture and identification of Salmonella

Carcass swab samples were homogenized by shaking manually with 10 mL of sterile peptone water (Merck, Darmstadt) and transferred to Selenite Cystine Broth (Merck, Darmstadt) prior to inoculation onto xylose–lysine deoxycholate agar (Oxoid, England). The plates were incubated under aerobic atmosphere at 37 $^{\circ}$C and examined after 24 h. Typical colorless colonies on MacConkey agar and pink to red colonies on xylose–lysine deoxycholate agar were picked and further identified through a series of

biochemical tests as per standard methods[5].

2.4. Enumeration of aerobic mesophilic bacteria

For aerobic mesophilic count, ten–fold dilutions of the homogenized carcass swab samples were pour plated in triplicate onto plate count agar (Oxoid, England). The plates were incubated at 35 °C for 48 h and the colonies were counted and recorded as CFU/cm^2 of carcass[6].

2.5. Assessment of risk factors

Data on the hygienic practices of the slaughter–men and the sanitation conditions of slaughtering area were collected with observation checklist.

2.6. Data analysis

Data were analyzed using SPSS version 20.0 and Epi Info version 3.3. The isolation rate of *Salmonella* species was calculated by dividing the frequency of positive samples by the total number of samples examined. Aerobic mesophilic counts were expressed in log 10 CFU/cm^2. *Chi*–square test was used to determine the association between the isolation rates of *Salmonella* species and handling practices of the slaughter men. Odds ratios were computed to measure the strength of association. Values were considered to be statistically significant when $P<0.05$.

3. Results

3.1. Isolation rate and distribution of Salmonella species

Out of 300 cattle carcass samples collected, 23 (7.6%) showed positive results for *Salmonella* species. Of these positive carcass samples, 6 (6%) were from the hind limb, 10 (10%) from the abdomen and 7 (7%) were from the neck region (Table 1).

Table 1
Distribution of *Salmonella* sppecies from cattle carcasses, Bahir Dar, Ethiopia, 2013.

Location of carcass	No. of carcass tested	No of samples tested positive for *Salmonella*	Percent (%)
Hind limb	100	6	6.0
Abdomen	100	10	10.0
Neck	100	7	7.0
Total	300	23	7.6

Relatively higher prevalence of *Salmonella* were detected in the abdomen than the neck and hind limb. Out of 23 *Salmonella* isolates, 11 (47.8%), 9 (39.1%) and 3 (13.1%) were *Salmonella* group A, *Salmonella arizonae* and *Salmonella typhi*, respectively (Table 2).

Table 2
Number and percentage of *Salmonella* species isolates from cattle carcasses, Bahir Dar, Ethiopia, 2013.

Subspecies	Number of isolates	% of isolates
Salmonella group A	11	47.8
Salmonella arizonae	9	39.1
Salmonella typhi	3	13.1
Total	23	100.0

3.2. The association between prevalence of Salmonella and hygienic practice of slaughter men

As presented in Table 3, from 100 cattle, 89 (89%) were slaughtered by slaughter men that wore garment.

Table 3

Association between isolation rate of *Salmonella* and hygienic practices of slaughter–men in Bahir Dar, Ethiopia, 2013.

Characteristics		Positive No (%)	Negative No (%)	Total No (%)	χ^2	P value*
Wearing garment	Yes	17 (19.1)	72 (80.9)	89 (89)	6.94	0.0080
	No	6 (54.5)	5 (45.5)	11 (11)		
Wearing boots	Yes	14 (18.9)	60 (81.1)	74 (74)	2.68	0.0880
	No	9 (34.6)	17 (65.4)	26 (26)		
Washing hands with soap before starting slaughtering	Yes	6 (37.5)	10 (62.5)	16 (16)	2.16	0.1300
	No	17 (20.2)	67 (79.8)	84 (84)		
Washing hands after separating intestinal contents	Yes	8 (14.5)	47 (85.5)	55 (55)	4.93	0.0200*
	No	15 (33.3)	30 (66.7)	45 (45)		
Washing carcass during slaughtering	Yes	11 (45.8)	13 (54.2)	24 (24)	9.29	0.0020
	No	12 (15.8)	64 (84.2)	76 (76)		
Washing knife before beginning slaughtering	Yes	17 (18.9)	73 (81.1)	90 (90)	8.58	0.0001
	No	6 (60.0)	4 (40.0)	10 (100)		
If the floor is sanitized	Yes	16 (17.8)	74 (82.2)	90 (90)	13.86	0.0001
	No	7 (70.0)	3 (30.0)	10 (10)		

*: Statistically significant at *P*<0.05.

The remaining 11 (11%) cattle were slaughtered by slaughter men without wearing garment and 6 (54.5%) of the *Salmonella* species were isolated from the cattle slaughtered by men without wearing garment. There was statistically significant association between wearing garment during slaughtering and the isolation rate of *Salmonella* in cattle carcasses (*P*=0.0080). As shown in Table 4, carcass samples from cattle slaughtered without wearing garment had five times risk of contamination with *Salmonella* (95% *CI*: 1.38–18.63).

Table 4

Summary of regression analysis on the isolation rate of *Salmonella* and hygienic practice of slaughter–men in Bahir Dar abattoir Ethiopia, 2013.

Characteristics		Number of samples	Positive No (%)	Odds ratio	Confidence interval	P value*
Wearing garment	Yes	89	17 (19.1)	5.08	1.38–18.00	0.0080
	No	11	6 (54.5)			
Washing hands after separating intestinal contents	Yes	55	8 (14.5)	2.93	1.11–7.77	0.0200
	No	45	15 (33.3)			
Washing carcass during slaughtering	Yes	24	11 (45.8)	4.51	1.64–12.42	0.0020
	No	76	12 (15.8)			
Washing knife before beginning slaughtering	Yes	90	17 (18.9)	3.15	0.82–12.05	0.0001
	No	10	6 (60.0)			
If the floor is sanitized	Yes	90	16 (17.8)	10.72	2.5–46.3	0.0001
	No	10	7 (70.0)			

*: Statistically significant at *P*<0.05.

The study revealed that 55 (55%) cattle were slaughtered by slaughter men that had the habit of hand–washing after separation of the intestinal content during slaughter, and

45 (45%) were slaughtered by slaughter men that did not have the habit of hand–washing after separation of the intestinal content. Fifteen (33.3%) *Salmonella* species were isolated from the cattle slaughtered by slaughter–men that did not have the habit of hand–washing after eviscerating the intestinal content. The habit of hand–washing after separation of intestinal contents was significantly associated with the isolation rate of *Salmonella* (*P*=0.0200). Carcass samples processed with hand–washing were less likely to be contaminated than those processed without washing (odds ratio 2.93, 95% *CI*: 1.11–7.77).

With regard to the habit of carcass washing during slaughter, 76 (76%) of cattle carcasses were not washed during slaughtering, of which 12 (15.8%) were positive for *Salmonella* species. However, the remaining 24 (24%) cattle carcasses were washed during slaughter, of which 11 (45.8%) were positive to *Salmonella* species. Carcass washing during slaughtering had a statistically significant association with the isolation rate of *Salmonella* in slaughtered cattle (*P*=0.0020). Carcass samples obtained without washing during slaughtering had 4.5 times greater chance of contamination with *Salmonella* than those processed with washing the carcass (95% *CI*: 1.64–12.42).

Ninety (90%) of the cattle were slaughtered by clean and washed knife, but the remaining 10 (10%) cattle were slaughtered without washing the knife. From this result, 17 (73.9%) of *Salmonella* species were isolated from the cattle which were slaughtered with washed knife. On the other hand, 6 (26.1%) of *Salmonella* species were isolated from the cattle slaughtered without washing the knife. It was observed that washing the knife before slaughtering has a statistically significant association with the isolation rate of *Salmonella* (*P*=0.0001). Carcass processed with unwashed knife has 3.15 times chance of contamination than that processed with washed knife (95% *CI*: 0.82–12.05).

In this study, 90 (90%) of cattle were slaughtered on clean and sanitized slaughter floor. Of these, 16 (69.6%) were *Salmonella*–positive. In contrast, 10 (10%) of cattle were slaughtered in on unsanitized slaughter floor, of which 7 (30.4%) were *Salmonella*–positive. The result showed that cattle slaughtered on sanitized slaughter floor were less likely to be contaminated with *Salmonella* (odds ratio 10.72, 95% *CI*: 2.5–46.3).

3.3. Aerobic mesophilic count

The mean aerobic mesophilic count of cattle carcass surfaces was 5.39 log CFU/cm^2 and ranged from 3.50–6.13 log CFU/cm^2. The mean aerobic mesophilic count of the hind limb, abdomen and neck were 5.38, 5.47 and 5.33 log CFU/cm^2, respectively (Table 5).

Table 5

The mean and range of aerobic mesophilic counts (log CFU/cm^2) on slaughtered cattle carcass surfaces in Bahir Dar slaughterhouse, Ethiopia, 2013.

Carcass locations	No. of carcass tested	Mean±SD	Minimum	Maximum	P value
Hind limb	30	5.38±0.70	3.61	5.87	
Abdomen	30	5.47±0.69	3.50	6.01	0.59
Neck	30	5.33±0.74	3.54	6.13	

SD=standard deviation.

4. Discussion

4.1. Isolation rate and distribution of Salmonella species

According to the study conducted in USA, the midline, neck and hind portion of the carcass have been found to be heavily contaminated areas[2,7]. The result of the current study is higher than the reports from slaughtered cattle in Ethiopia[3], in Khartoum Sudan (4.2%)[8], while the isolation rate obtained from this study was lower than 37.3% prevalence recorded from a study done in Namibia (10.9%)[9] and Algeria (10%)[10]. The difference could be due to seasonal factors in contamination of beef with *Salmonella* species[11]. Other causes for this variation are associated with stress during transportation to the slaughterhouse, hygienic conditions of holding pens, processing practices, abattoir facilities and employee's hygiene and practices[3,7].

The distribution of *Salmonella* species among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestation, and production systems[12]. Most cases of salmonellosis are caused by food infected with *Salmonella enterica*, which often infects cattle and poultry, though other animals such as domestic cats and hamsters have also been shown to be sources of infection to humans[13]. In this study the detection of *Salmonella* groups A and *Salmonella tyhpi* indicates that the contamination is of human origin and result of poor personal hygiene during handling and processing of the meat products[11]. *Salmonella enterica* subspecies *arizonae* is naturally found in reptiles but also causes outbreaks of salmonellosis in turkeys and sheep and can produce both enteritis and serious disseminated disease in humans[14]. Reptiles frequently harbor subspecies *arizonae* as a commensal subspecies, but pathological responses to infection have also been reported, and the bacteria seem to be capable of vertical transmission through infection of oviduct. Many human and other animal infections can be traced to contact with reptiles or ingestion of various reptile products, particularly from rattlesnakes[14].

4.2. Association between prevalence of Salmonella and hygienic practices of slaughter–men

The important factors of *Salmonella* contamination includs different subspecies and slaughter men wearing garment or not. These results are confirmed by the study of Whyte *et al*[15]. Similar study in Peshawar postulated that the control of pathogens including *Salmonella* on carcasses could be achieved by adopting standard dressing procedures[16]. According to Bloomfield *et al*[17], even wet–cleaning of clothes is a significant contaminant in disseminating *Salmonella* to different surfaces and the hands of the workers.

Hand–washing after separation of intestinal content has a statistically significant association with the isolation rate of *Salmonella* in slaughtered cattle carcasses (*P*=0.02). The present study contradicts the study conducted in other localities in which there was no statistically significant association between the evisceration with hand and carcass contamination[18]. On the other hand, when the carcass is opened and the viscera removed, spillage of rumen and intestinal fluids may contaminate the workers (or slaughter men)[7]. This study is in line with the study conducted by Bouchrif *et al*.[19] in that the leading source of contamination of carcasses by *Salmonella* is the evisceration step at the slaughterhouse. According to Abdalla *et al*[8], fecal matter was a major source of contamination and could reach carcasses through direct contact with workers without washing their hands.

The current study is in agreement with the study conducted in Ethiopia, which showed that the eviscerating knife was found to be significantly associated with carcass–contamination by *Salmonella*[18]. Similar finding was reported by Abdulla and his colleagues that there was significant relation between washing of knife and positive detection of *Salmonella*[16]. This study is in line with the study conducted in Khartoum North, Sudan[8]. Study conducted in India also showed that cutting equipments are the major contaminants of fresh meat in the slaughter plant[20].

The result showed that cattle slaughtered in clean and sanitized slaughter floor had statistically significant association with the prevalence of *Salmonella*. This result is comparable with the study conducted by Abdalla *et al*.[8] which explained that the slaughterhouse floor has significantly (*P*<0.05) higher degree of *Salmonella* contamination. So, cleaning and sanitizing of the slaughterhouse were a key component of good practices at slaughterhouse, and can confer significant benefits in terms of reducing the incidence of *Salmonella* contamination to the carcass. Similarly, the contamination of cattle carcass also originates from the floor where animals are being stunned and bled and this has been reported in other studies as well[3]. The slaughtering–plant operations generally amplify the level of bacterial contamination as observed in this study and the study conducted in the United Arab Emirates[21]. This study is also supported by the study conducted in India, which showed that floors, platforms and walls on most occasions are contaminated due to microorganisms including *Salmonella* brought in by animals along with hides and faces and also through blood droppings and rupture of viscera. This significantly contaminates the cattle carcass[22]. From this result, the present study found that carcass washed during slaughter had a statistically significant association with the prevalence of *Salmonella* in slaughtered cattle (χ^2=9.29, *P*=0.002).

Feces, as well as soil adhering to the animal, are carried into the abattoir on the hair, hide, hooves and tail of the animal[7]. In addition to feces, the occurrence of *Salmonella* on the external surfaces of cattle carried into a slaughterhouse can serve as an indication of contamination that potentially could be transferred to carcass surfaces during the dehiding process[3]. It is also subjected to contamination by the digestive flora during eviscerating[10]. So, carcass washing plays a great role in reducing the prevalence of *Salmonella* at the slaughterhouse. This study was supported by the study conducted in Iran. It was

stated that the washing procedure in the slaughterhouse decreases *Salmonella* contamination on the cattle carcass[23]. In the current study no cattle carcass was washed by warm water.

4.3. Aerobic mesophilic count

On the one hand, only 14.4% of cattle carcass samples was at a good standard. About 21.1% was acceptable and 64.4% was unacceptable microbiological level based on the standards of Food and Agricultural Organization[24]. The high level of aerobic mesophilic counts after washing of cattle carcasses in this study is in agreement with that reported by Nouichi and Mossadak[10] in Algeria. Similarly, Adzity *et al*.[9] documented a mean of 5.76 log CFU/cm^2 in Ghana. On the other hand, the result is higher than the mean on cattle carcasses reported by Paszkiewicz and Renatapyz[25], and Abdalla *et al*.[8] from Sudan with a mean at shoulder, neck and brisket site.

A study by Bryant *et al*.[26] revealed that the workers' hands and the equipment were the sources of meat contamination during the slaughtering process. Particularly, the high aerobic mesophilic count in this study might be due to the contamination of fresh meat by unclean utensils, and dirt[27]. The presence of aerobic mesophilic count in large quantity is explained also by contact with unclean slaughtering area, and bad evisceration practices, often resulting in rupture of the gastrointestinal tract, and especially hide-to-carcass contamination transfer during slaughtering[10]. The environmental exposure to contaminants, the health of the cattle itself, and stressful situations, which may increase microbial shedding in cattle such as disrupted access to feed and water, transport, handling and contact with other animals could be the factors of significance in contamination. Processing stations and slaughter practices are also potential sources of carcass-contamination in the slaughterhouse[7].

This study shows that most carcasses from slaughtered cattle in Bahir Dar abattoir were of unacceptable bacteriological quality. Unlike other studies, *Salmonella* group A appears to be the most prevalent in cattle carcasses in the present study and the highest contamination was recorded in the abdomen among carcass sites. Therefore, human and the intestinal content are main sources of contamination. It is therefore recommended that good hygienic and sanitary conditions should be observed in the abattoir during slaughtering operations.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Comments

Background

In countries like Ethiopia where consumption of raw meat is a common practice, the risk of pathogenic contamination and consequent infections in humans is very high. Even half-cooked meat can carry considerable proportions of contaminations. Of the many pathogenic bacteria found in cattle, *Salmonella* is known for its toxicity, causing food-poisoning, gastroenteritis, and typhoid fever. Thus, examining its prevalence in cattle carcasses and the causative factors thereof has substantial importance towards suggesting the corrective measures.

Research frontiers

The study has been performed by taking a reasonably large sample of 300 cattle carcasses, giving the distribution of *Salmonella* in body areas like hind limbs, abdomen and neck, notwithstanding that the bacterium is primarily an intestinal pathogen. The samples were apparently collected and analyzed under hygienic conditions without giving room for further contaminations. The experiments including the cultures and identification of *Salmonella* species were obviously performed by following standard and up-to-date methodology, which confers reliability on the results obtained on the three species of *Salmonella*.

Related reports

The study has compared its results with those of a number of other studies performed in different countries and other regions of Ethiopia on the prevalence of *Salmonella* and salmonellosis. Some of the items chosen, for example, the selection of hind limbs, abdomen and neck for analysis, were logically based on the studies performed by other investigators. This nevertheless does not detract the scientific value of the present study.

Innovations and breakthroughs

The central idea of this investigation stems from the observation that the distribution of *Salmonella* species among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestations, and production systems. In contrast to the studies of others, the present study reports that *Salmonella* group A is most prevalent in cattle carcasses, which is a notable finding of this study.

Applications

The study takes into account the distribution of *Salmonella* species in the cattle carcasses in the local abattoir in Bahir Dar town. The results and the conclusions thereof could be very well applicable in a generalized way to other places in Ethiopia and other countries, where similar practices are followed. Consequently, the corrective measures suggested by the authors towards improving the bacteriological quality of the meat from cattle carcasses find general acceptance at other places as well.

Peer review

Studies of this kind are especially important in countries

like Ethiopia where hygienic practices fall below the expected level, and consequently the prevalence of bacterial infections from consumption of raw and half–cooked meat is considerably high and will be a risk factor from the point of view of personal and public health. The authors have carefully chosen their investigation and executed it successfully. The methodology followed meets the standards expected, and the results obtained are apparently reliable. The discussions made on the results are sound, and the conclusions drawn are appropriate. The corrective measures suggested by the authors should find general acceptance. The literature survey is thorough, with quite a few recent and appropriate references included.

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