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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Salmonella and risk factors for the contamination of cattle carcass from abattoir of Mekelle City, Ethiopia

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Abstract: The objective of this study was to assess the incidence of *Salmonella* species and identify the risk factors for the presence of *Salmonella* and *E.coli* on cattle carcasses of Mekelle abattoir. A total of 96 swab samples were collected from hind limb, abdomen and neck of 32 randomly selected cattle and taken in an icebox to laboratory for microbial analysis. Analyses were done within 1 h of sample collection. Swab samples were homogenized by manually shaking with 10 ml of sterile peptone water and transferred to Selenite Cystine Broth prior for inoculation onto xylose-lysine deoxycholate agar. The plates were incubated under aerobic atmosphere at 37°C and examined after 24 h. The mean aerobic count of hind, abdomen and neck found were 2.40, 2.37 and 2.33 with overall mean of log CFU/100 cm^2 2.37. Of the 32 oxen/cows included in the study, 4 (12.5%) of them were found positive for the presence of *Salmonella*. The occurrence of *Salmonella* in the neck was found to be higher compared to other parts of the carcass. Of the 96 swab samples, 18 swab samples from 6 cattle neither their hind limp and abdomen nor their neck was positive for the presence of *E.coli*. However, 19 hind limb, 18 of the abdomen and 18 of neck were found to be positive for the presence of *E.coli*. The bacteriological swab tests of the carcass parts also showed that the meat contained

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PUBLIC INTEREST STATEMENT

A food-safety hazard is anything in food that can hurt consumers' health. There are three major types of hazards (Chemical, Physical and Biological). Biological hazards are living organisms, including viruses, bacteria, protozoa, molds and parasites, that have the ability to infect or produce toxins harmful to health. Studies found that food-borne disease was common, affecting around one in three to one in six people a year and resulted in a high burden of disease. Norovirus, *Campylobacter, E.coli* and Non-typhoidal *Salmonella* are the key global causes of diarrheal diseases. Assessment of food safety and training community how to handle, process and consumption of food hygienically support national economies, trade and tourism, contribute to food and nutrition security and underpin sustainable development.

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aerobic mesophilic bacteria and pathogenic microorganisms which show lack of hygienic condition and improper handling of the cattle before slaughtering.

Subjects: Food Chemistry; Sensory Science; Dairy Science

Keywords: Salmonella; carcass; E.coli; neck; abdomen

1. Introduction

Meat chemical composition is likely for the growth of many of microbial populations which makes raw meat to be one of the way of food-borne contaminations in people (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Scallan et al., 2011). The real quantity of food-borne contagions related to meat is challenging to evaluate exactly, mainly because only a minor quantity of illness cases is officially reported especially in growing nations. Uncooked meat from slaughterhouse exists in open-air local vending shops without proper temperature management, which is bought by households and also incompletely cooked crushed meat is consumed in restaurants. A periodic surveillance on the prevalence of zoonotic pathogens such as *Salmonella* and their risk factors in slaughtered food animals is essential to control the spread of the pathogen and infection in man through con-taminated carcass (Molla, Mesfin, & Alemayehu, 2003). This is required to propose the acceptance of the carcass in relation to the standards.

Salmonella species are top gastroenteritis in numerous nations, and salmonellosis is a major food-safety hazard (Amwayi, Kikuvi, & Muchiri, 2010). Food from animals like cattle might hold *Salmonella* at slaughter and can, at the same time, be the basis of contagion and endow with an opening for entry of the pathogen into the food products. This means that the existence of *Salmonella* in slaughtered cattle and slaughter house surroundings and the possible cross-contamination of carcasses and safe to eat organs can cause food-safety risks (Sibhat et al., 2011). Next to malaria and respiratory infectivity, diarrheal illnesses are one of the key reasons of morbidity and mortality particularly for children in growing nations. Among those diseases, salmonellosis is considered as the most common food-borne illness in developing countries as well as in the industrialized ones, although prevalence amount differs relating to the countries (Motarjemi et al., 1995). The improvement and the increase in tolerance to antibiotics in these pathogens are a main concern in community health. It is commonly customary that, in growing nations various multi-resistant *Salmonella* are of animal origin and obtain their resistance in animals before being conveyed to human in the course of the food succession (Threlfall, 2002; White et al., 2001). In the United States, a study showed that 20% of 200 samples of ground chicken (35%, *n* = 51), beef (6%, *n* = 50), turkey (24%, *n* = 50) and pork (16%, *n* = 49) purchased at three retail store are infected by *Salmonella* (White et al., 2001). In meat production, the primary source of pollution of carcasses by Salmonella is the evisceration step at the abattoir (Samuel, O'Boyle, Mathers, & Frost, 1980). About 20% of camel at slaughtering and 15% at the vendor's level of the meat samples were found positive for Salmonella in Ethiopia (Ejeta, Molla, Alemayehu, & Muckle, 2004). 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 of slaughter-ing house of Mekelle. Therefore, the objective of this study was to assess the incidence of *Salmonella* species and identify their risk factors on cattle carcasses of Mekelle abattoir.

2. Materials and methods

2.1. Description of study area

A cross-sectional study was conducted from June 2016 to September 2016 to assess the isolation rate of *Salmonella species* in cattle carcass samples from Mekelle abattoir.

2.2. Sample collection

A total of 96 carcass (32 from each of hind limb, abdomen and neck) samples were collected from 32 randomly selected cows and/or oxen. About 100 cm^2 of cattle carcass surface around the hind limb, abdomen and neck was swabbed by wiping the cotton swabs on each sampling site, five times in both vertical and horizontal directions for 30 s using sterile surgical gloves (Amwayi et al., 2010). The swab samples were transported to Mekelle University Food Science and Postharvest Technology laboratory using an icebox within 1 h of collection. The swab samples were analyzed immediately for the presence of *Salmonella* and aerobic mesophilic counts.

2.3. Sample processing, culturing and identification of Salmonella

Carcass swab samples were homogenized by manually shaking with 10 ml of sterile peptone water (Merck, Darmstadt) and transferred to Selenite Cystine Broth prior to inoculation onto xylose-lysine deoxycholate agar. The plates were incubated under aerobic atmosphere at 37°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 (Cheesbrough, 2006).

2.4. Enumeration of aerobic mesophilic bacteria

For aerobic mesophilic count, 10-fold dilutions of the homogenized carcass swab samples were pour plated in triplicate onto plate count agar. The plates were incubated at 35°C for 48 h and colonies were counted and recorded as CFU/cm^2 of carcass (Adzitey, Teye, Kutah, & Adday, 2011).

2.5. Assessment of risk factors

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

2.6. Data analysis

Data were analyzed using GenStat 13th Edition (SP2). The isolation rate of *Salmonella* species were 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. Values were considered to be statistically significant when $p < 0.05$.

3. Result and discussion

3.1. Practical observation of Abergelle abattoir Private Limited Company

Abergelle international abattoir Private Limited Company has two alienated lines for cattle and Shoat slaughter. It's Lairage has enough pens for daily slaughtering, but the fasting period is not like the scientifically recommended which is 12–24 h prior to slaughtering Slaughter operations were performed on slaughter lines including separated wet areas and clean areas. After being stunned in a stunning box using a sharp knife (sticking), animals were attached by the right rear leg and directly (within 60 s) exsanguinated. Before skinning, head and hooves were detached. Skinning operations comprised manually performed pre-skinning and mechanized skinning by an upward-pulling hide puller. Before evisceration, carcasses were moved into separated clean areas. Evisceration involved slitting the belly, removal of the gut and removal of thoracic viscera. Carcasses were then split along the midline from back to front with a splitting saw. After trimming, meat inspection, weighing and grading, carcasses were washed with cold potable water to remove visual debris. The slaughter men are not aware of the standard handling of carcass because their education level is at elementary level. Postmortem inspection was done by veterinary doctors after the evisceration process. The average cattle slaughter in the abattoir is 100 per day.

4. Microbiological analysis

4.1. Total aerobic mesophilic count

Meat swab samples from different carcass parts named hind, neck and abdomen were analyzed for their aerobic mesophilic count and presence of different pathogenic microorganisms, namely, *Salmonella, Shigella, E.coli* and *Proteus Vulgaris*. The mean values of aerobic mesophilic count of different parts from oxen/cows are summarized in Table 1.

The overall mean aerobic mesophilic count of cattle carcass surfaces was log CFU/100 cm^2 2.37 and with a range of log CFU/100 cm^2 2.33–2.40. The mean aerobic mesophilic count of hind, abdomen and neck were 2.41, 2.37 and 2.33 log CFU/100 cm^2. When comparing the mean aerobic mesophilic count of the 10 oxen and/or cows, the uppermost CFU/100 cm^2 was recorded in hind of fifth Ox (2.555 ± 0.002) while the lowest were recorded from neck of the second Ox/cow (2.087 ± 0.002) (Table 1). Among the 10 oxen and/or cows, the aerobic mesophilic count from ox 1, 2, 4, 6 and 8 has similar count which is significantly different from

Table 1. Total aerobic mesophilic count of different parts of 10 oxen/cows	
Sample	**Mean ± standard deviation**
1H	2.237 ± 0.003^n
1N	2.280 ± 0.003^m
1A	2.387 ± 0.002^h
2H	2.138 ± 0.011^q
2N	2.087 ± 0.002^r
2A	2.383 ± 0.002^h
3H	2.466 ± 0.003^e
3N	2.162 ± 0.006^o
3A	2.381 ± 0.005^{hi}
4H	2.521 ± 0.002^c
4N	2.446 ± 0.002^f
4A	2.388 ± 0.003^h
5H	2.555 ± 0.002^a
5N	2.479 ± 0.053^d
5A	2.380 ± 0.006^{hi}
6H	2.420 ± 0.005^g
6N	2.310 ± 0.004^k
6A	2.388 ± 0.003^h
7H	2.449 ± 0.002^f
7N	2.151 ± 0.006^p
7A	2.373 ± 0.001^i
8H	2.381 ± 0.005^{hi}
8N	2.418 ± 0.004^g
8A	2.388 ± 0.005^h
9H	2.333 ± 0.004^j
9N	2.536 ± 0.001^b
9A	2.295 ± 0.001^l
10H	2.559 ± 0.002^a
10N	2.478 ± 0.001^d
10A	2.381 ± 0.005^{hi}

Means with different superscript letters are significantly different ($p < 0.05$).

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Table 2. Occurrence of Salmonella in different carcass parts from Mekelle abattoir			
Carcass location	No. of carcass tested	No. of sample tested positive for *Salmonella*	Percent (%)
Hind limp	32	2	6.25
Abdomen	32	2	6.25
Neck	32	3	9.37
Total	96	7	**7.29**

the total aerobic mesophilic count from oxen 3, 5, 7, 9 and 10 at (p < 0.05). Study accompanied in Unites States shows the midline, neck and hind portion of the carcass have been found to be heavily contaminated areas (Alemayehu, Molla, & Muckle, 2003; Amwayi et al., 2010).The aerobic mesophilic count of this study is lower than the reports from slaughtered cattle in Bahir Dar (Muluneh & Kibret, 2015). Based on the standards of Food and Agricultural Organization, the microbiological level of this study is acceptable (Carlson et al., 2008). The environmental disclosure to pollutants, the well-being of the cattle itself and stressful conditions which can increase microbial shedding in cattle such as unsettled access to feed and water, transport, handling conditions and contact with other animals could be factors of significance in contamination. Processing places and slaughtering techniques are likewise possible causes of carcass contamination in the slaughterhouse (Arthur et al., 2004). Even though we did not follow the chain of the oxen/cows slaughtered in the abattoir fourth-year students of Food Science and Post-harvest Technology have examined, the total aerobic mesophilic bacterial count of meat from different butchery in different kebeles and the count ranges from 7.27 to 8.3 log cfu/g. Meat sample from kebelle 15 for third butchery exhibited the highest total bacterial count of 8.3 log cfu/g and meat from kebelle 15 for first butchery exhibited the lowest total bacterial count of 7.27 log cfu/g. All the samples had total bacterial count of more than 5.00 log cfu/g which is above the safe limit because food were classified as acceptable if the bacterial count was less than or equal to 5 log10 CFU g^{-1} (NSW Food Authority, 2012)

4.2. Isolation rate and distribution of pathogenic microorganisms
The 96 carcass swab samples obtained from different parts of oxen/cows were analyzed for the presence of pathogenic microorganisms. Out of the collected samples 5 (5.2%), 2 (2.08%), 1 (1.04%), 1 (1.04%), 55 (57.29%), 61 (63.54%) of the 96 carcass swab samples were positive for the presence of *Salmonella Typhi, Salmonella choleraesuis, Shigellasonnei, Shigellaflexneri, E.coli* and *Proteus Vulgaris* respectively (Tables 1–4). The presence of high microbiological contamination displays the poor hygienic quality of carcasses, which in turn generates hazards for public health and food safety.

Out of the 32 oxen/cows, 4 (12.5%) showed positive result for the presence *Salmonella*. The occurrence of *Salmonella* has been found to be higher in neck compared to other parts of the carcass (hind limp and abdomen). The prevalence of *Salmonella* in meat carcass from Mekelle abattoir seems to be low but contaminated bovine meat remains a significant risk for *Salmonella* infection in humans particularly for people consuming more beef because the ample protein and fat content of foods like meat was stated to safeguard the bacterium against the gastric acidity (Birk et al., 2012; Blaser & Newman, 1982; Kothary & Babu, 2001). This warns that the consumption of contaminated meat, even with a low number of pathogens, would cause a significant risk of infection and/or intestinal colonization in humans. As for other bacterial pathogens, the lowest number of *Salmonella* capable of initiating illness is difficult to fix as it depends on several factors including (but not limited to) the food matrix, the host susceptibility and the virulence factors of the pathogen (McEntire, Acheson, Siemens, Eilert, & Robach, 2014). However, recent studies using outbreak data indicate that doses as low as 36 colony-forming units can cause illness in humans (Teunis et al., 2010). This infective dose would be qualified as "low" comparatively to food-borne

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Table 3. Occurrence of E.coli in different carcass parts from Mekelle abattoir			
Carcass location	No. of carcass tested	No. of sample tested positive for *E.coli*	Percent (%)
Hind limp	32	19	59.37
Abdomen	32	18	56.25
Neck	32	18	56.25
Total	96	55	**57.29**

Table 4. Occurrence of Shigella in different carcass parts from Mekelle abattoir			
Carcass location	No. of carcass tested	No. of sample tested positive for *Shigella*	Percent (%)
Hind limp	32	0	0
Abdomen	32	1	3.1
Neck	32	0	0
Total	96	1	**1.04**

Except one ox and/or cow, all the samples are free of shigella.

Table 5. Occurrence of proteus Vulgaris in different carcass parts from Mekelle abattoir			
Carcass location	No. of carcass tested	No. of sample tested positive for *Proteus Vulgaris*	Percent (%)
Hind limp	32	20	62.5
Abdomen	32	20	62.5
Neck	32	21	65.62
Total	**96**	**61**	**63.54**

pathogens such as *Vibrio cholera* that require doses as high as 10^4–10^8 cells to cause infection in humans (Kothary & Babu, 2001).

Out of the 96 samples, in 6 (18.75%) oxen and/or cows, neither their hind limp and abdomen nor their neck, is positive for the presence of E.coli, whereas from the remaining 19 (59.37%), hind limp, 18 (56.25%) of abdomen and 18 (56.25%) of neck are highly positive for the presence of E.coli (Table 3). The infective dose for pathogenic *E coli* is known to be "low". Coia (1998) reported contamination levels as low as two organisms per 25 g in food and environmental samples implicated in VTEC O157 outbreaks. Because of the low infective dose, the contamination limit for these pathogens has been fixed to the absence in 25 g of meat preparations intended to be eaten raw (European Commission, 2005). The isolation rate of *E.coli* from 12 butcheries from different kebelles of Mekelle was very high. About 91.7% was positive and only one sample was negative (8.3%).

Except one ox/cow 31 of the oxen and/or cows were free for the presence of **Shigella,** while 26 (81.25%) were positive for the presence of **Proteus Vulgaris** in which 20 (62.5%) from hind, 20 (62.5%) from abdomen and 21 (65.6) were from neck (Tables 4 and 5).

5. Conclusion
The bacteriological swab tests of the carcass parts confirmed that the meat contains aerobic mesophlic bacteria and pathogenic microorganisms which show lack hygienic condition and improper handling of the cattle before slaughtering. Pathogens such as *Salmonella* spp., *Shigella*

spp. and *Escherichia coli* and *Proteus Vulgaris* were the main identified organisms in the carcass. It is concluded that the sanitary conditions of the abattoir and handling of animals before slaughtering required strict follow-up for the provisions of sanitary codes. Periodic sanitary-hygienic evaluation and training the slaughter men should be strengthened to reduce public health hazards.

Availability of data and materials
This research has been investigating the incidence of pathogenic microorganisms on carcass and its risk factors which will give insight to the community how to handle carcass hygienically.

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Authors' contributions
Study conception, design and acquisition of data were done by Teklebrhan WT Analysis and interpretation of data and drafting of manuscript: critical revision Lijalem TW, Hagos HK., Kibrom AS. and Getachew TA. All authors read and approved the final manuscript.

Consent for publication
All authors (Getachew Tafere Hagos Hailu Kassegn, Lijalem Tareke and Teklebrhan Welday, Kibrom Abera) agreed to publish the manuscript to this journal (Cogent Food and Agriculture).

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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Citation information
Cite this article as: Salmonella and risk factors for the contamination of cattle carcass from abattoir of Mekelle City, Ethiopia, Teklebrhan Welday Atsbha, Lijalem Tareke Weldeabezgi, Kibrom Abera Seyoum, Getachew Tafere & Hagos Hailu Kassegn, *Cogent Food & Agriculture* (2018), 4: 1557313.

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