Salmonella prevalence in cattle slaughtered in selected abattoirs of Amhara National Regional State, Ethiopia

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Abstract

Salmonella can spread more readily since they are present in humans, animals, and the environment. Some *Salmonella* serotypes exert huge health and economic impacts due to their virulence or antibiotic-resistance traits. This study aimed to detect *Salmonella* and determine its prevalence in slaughtered cattle of selected towns` abattoirs in the Amhara region. The study was cross-sectional, and a systematic random sampling technique was used to select the study animals. A total of 480 animals` feces, carcass swabs, liver and intestinal lymph node samples were collected for this study. Bacteriological and polymerase chain reaction (PCR) tests were used. The *Salmonella* prevalence at the animal level was 8.33% (10 out of 120) (95% CI: 4.07% - 14.79%). At sample level, 11 isolates out of 480 samples were found positive for *Salmonella*, with a prevalence of 2.29% (95% CI: 1.15% - 4.06%). The PCR amplifications gave products of 496 bp for 11 isolates by gel electrophoresis and were consequently confirmed as *Salmonella.* To know the possible source of contamination, water used for carcass washing and sanitation, as well as swab samples from butchers' hands, coats/aprons, boots, and all slaughtering materials, were tested for *Salmonella*. All of these samples were found to be negative for *Salmonella*. A significant number of *Salmonella* was identified in slaughtered cattle in this study. Emphasizing the significance of enhancing hygienic practices prior to, during, and after the slaughtering process is of utmost importance. Sources of threats for *Salmonella* contamination, including abattoirs, should be given more attention to help them establish and maintain strict hygienic measurements.

Keywords: Abattoir; Cattle; Prevalence; *Salmonella*.

Introduction

Salmonella is one of numerous foodborne pathogens that could pose a significant threat to global food safety. About 2700 serotypes (serovars) have been identified so far (Dnog *et al*., 2020); the majority of them can produce disease in animals and humans. The genus is responsible for an array of diseases, such as typhoid fever and salmonellosis (a variety of illnesses, including gastroenteritis), which cause public health issues globally (Ryan *et al*., 2017).

Salmonella contamination of meat constitutes a significant risk to the public's health as it can result in fatal illnesses. The bacteria are among the common pathogens in food animals, establishing an asymptomatic reservoir that makes it challenging to control and eradicate (Salman and Steneroden, 2014).

Animal meat is the primary reservoir for many foodborne pathogens. Meat products, mainly fresh meat, have a small number of microorganisms. However, when exposed to favorable growth conditions, they can make the meat unusable for human consumption. The presence of pathogenic bacteria like *Salmonella*, *E. coli* O157:H7, and *Listeria* or their toxic products make meat unsafe for human consumption, and lead to gastrointestinal diseases if ingested (WHO, 2015).

Salmonellosis is a significant cause of economic loss in farm animals because of the cost of diagnosis, treatment of clinical cases, cleaning and disinfection, control and prevention costs, and animal death (Pal *et al*., 2020). The globalization of the food supply, expansion of food businesses, changes in food eating habits, and the increased movements of people, animals, and goods within and between countries are bringing new food safety hazards and threats. Food safety is also facing a severe issue with the rising prevalence of foodborne pathogens that are resistant to antimicrobial drugs (Jaffee *et al*., 2018).

The issue of animal-origin foodborne diseases is still a significant threat in developing countries like Ethiopia due to inadequate hygienic standards, a habit of consuming raw meat, and undeveloped infrastructures. Salmonellosis is one of the most significant and common zoonotic illnesses. Since no practical steps have been taken to control it, the problem is highly present on the ground. The distribution of *Salmonella* has not been thoroughly studied and defined at the regional or national level. There is insufficient information regarding *Salmonella* contamination of carcasses during cattle slaughter. Thus, the present

study was conducted to estimate the prevalence and assess the distribution of *Salmonella* in slaughtered cattle of selected abattoirs of the Amhara region, Ethiopia.

Materials and methods

Study area

The study was carried out in abattoirs from selected towns in the Amhara region, namely Gondar, Bahir Dar, Debre Markos, Debre Tabor, Kombolcha, and Debre Birhan. The Amhara region is located in the northwestern part of Ethiopia between 9°20′ and 14°20′ North latitude and 36°20′ and 40°20′ East longitude. The region covers approximately 161,828.4 km^2 area. Based on the 2007 census of the Central Statistical Agency of Ethiopia (CSA), the Amhara region has a population of 17,221,976 (8,641,580 men and 8,580,396 women) (CSA, 2007). The 2016/17 CSA agricultural sample survey estimated the region's ruminant livestock population at 15.98 million cattle (representing 26.8% of Ethiopia's total cattle population), 9.79 million sheep (representing 31.8% of Ethiopia's total sheep population) and 6.08 million goats (representing 20.1% of Ethiopia's total goat population) (CSA, 2017).

Study animals

The study animals were apparently healthy slaughtered cattle from selected abattoirs in the Amhara region. The animals were mostly bought from nearby small towns and rural marketplaces and transported to the abattoirs on foot or open trucks made for the transportation of other goods. Both intensive and semi-intensive cattle husbandry systems are practiced in the areas. Those under semi-intensive management graze pasture, and they are also fed hay throughout the long dry season when the pasture is in short supply. Animals under intensive management are usually fed with hay and concentrate. Most of the cattle slaughtered in the abattoirs were adult males of the local zebu breed. All cattle in the study abattoirs ready for slaughtering during the study period were the primary sources of the test samples. Water sources that were utilized for carcass washing and sanitation in the abattoir, all materials that were utilized for slaughtering and inspection, and butchers' hand and safety dressings were also sampled to determine whether they could be the sources of contamination or not.

Study design and sampling technique

A cross-sectional study was conducted from February 2022 to January 2023. Study animals were selected using a systematic random sampling method using a three-animal sampling interval, with the first animal to be sampled on a particular sampling day selected randomly among the first three animals on the slaughter line.

Sample size determination

The sample size required for this study was determined according to Thrusfield (2018). The expected prevalence of 7.6% (Muluneh and Kibret, 2015) for *Salmonella* in the study area, a 95% confidence interval (95% CI), and a 5% level of precision were considered to calculate the sample size.

$$n=Z^2 \times P_{exp} (1-P_{exp})/d^2$$

$$n=(1.96)^2 \times 0.076(1-0.076))/0.05^2$$

$$n = 108$$

where n is the required sample size, Z is 1.96, P_{exp} is the expected prevalence, and d is the desired absolute precision of 0.05. Therefore, the calculated sample size was 108. However, twelve animals were purposefully added in order to improve the study's precision and preserve the distribution balance during sampling. So, a total of 120 animals were selected for this study. The entire sample size was distributed proportionally among the study abattoirs. Accordingly, 20 animals from each abattoir were selected and examined for *Salmonella*. The number of sampled cattle on a particular day could be from 2 to 10, depending on the number of cattle presented for slaughter.

Sample collection and transportation

Four types of samples were collected from each animal: feces (before slaughter), carcass swabs (CS), mesenteric lymph nodes (MLN), and liver. As a result, 480 samples were collected from the 120 study animals, 80 samples each from the six abattoirs.

About 25 g of fecal samples were collected directly from the rectum of each selected cattle before the animal was slaughtered using disposable sterile plastic gloves. Carcass swab samples from the flank, brisket, and rib area were col-

lected using a sterile cotton-tipped swab rubbed on the carcasses firmly. The swab was then inserted into a sampling container containing 10 ml buffered peptone water (BPW) (Oxoid, England) after cutting off the part of the stick that was in contact with the hand.

Small parts of the liver, about 25 g, adjacent to the gallbladder, were collected from each study animal. Mesenteric lymph nodes were also collected by dissecting the mesenteric fat using a sterile blade and forceps.

A total of 60 water samples (each 10 ml) from the sources of water, mainly pipe and tanks, which were used for washing hands and cleaning equipment (knives, hooks, and carts), and swab samples from butchers' hands (n = 65), coats/aprons (n = 65), boots (n = 65) and equipment used in the slaughtering processes (84 knives, 50 hooks, 26 carts) were aseptically collected for the test. All samples were collected purposely during each sampling day.

All sample types were collected aseptically and transferred immediately to sterile labeled universal bottles containing BPW. A cold chain transport method was used, keeping the collected samples in ice boxes containing ice packs until arrival at the laboratory. Samples were transported to the Veterinary Microbiology Laboratory, College of Veterinary Medicine and Animal Health Sciences, University of Gondar, within 30 minutes to 24 hours, depending on the distance of the area of sample collection. Upon arrival at the laboratory, the samples were processed immediately.

Isolation and identification of *Salmonella*

The technique recommended by the International Organization for Standardization, ISO 6579 (ISO, 2013), was employed to isolate and identify *Salmonella* organisms. Carcass swab materials and fecal samples were homogenized in 225 ml BPW. A 25 g tissue samples (liver and MLN) that had been weighed by digital balance (Heraus, Germany) were placed in a sterile Stomacher bag and homogenized in 225 ml of BPW at a ratio of 1:9. The sample suspension was prepared in a peristaltic homogenizer (Stomacher 400, UK) for 2 minutes. The samples were incubated for 18 to 24 hrs at 37 ˚C. Following this, 1 ml and 0.1 ml of the pre-enrichment broths were transferred aseptically into 10 ml of Muller Kauffmann Tetrathionate (MKTT) (Merck, Germany) and 10 ml of Rappaport-Vassiliadis (RV) broth (HiMedia, India), mixed, and then were incubated for 18 to 24 hrs at 37 ˚C and 42 ˚C, respectively. Following incuba-

tion, a loop-full of each culture was streaked onto the surface of xylose lysine deoxycholate (XLD) (HiMedia, India) and brilliant green agar (BGA) (Oxoid, England) medium and incubated at 37 ˚C for 24 to 48 hrs. The XLD and BGA plates were examined for the presence of suspect *Salmonella* colonies. Then, plates with colonies with slight growth and plates with no typical *Salmonella* colonies were re-incubated for an additional 18 to 24 hrs and re-examined. The formation of red colonies with black centers and of pink colonies with a red zone was inspected on XLD and BGA plates, respectively. Colonies showing typical morphological characteristics of *Salmonella* were sub-cultured to get pure colonies for further biochemical and molecular identification (Mooijman *et al*., 2019). A similar procedure was applied for water and equipment swab samples.

The following biochemical tests were used for further identification of all suspected *Salmonella* colonies: lysine decarboxylase (LDC), indole, citrate, triple sugar iron (TSI) agar, and urease tests. All cultural media were products of HiMedia, an Indian company. The inoculated test tubes were incubated for 24 or 48 hrs at 37 °C to see changes in their biochemical properties (ISO,2002). Colonies of the biochemically confirmed isolates were sub-cultured in a nutrient broth to refresh them. They were delivered to the National Veterinary Institute, Molecular Biology Laboratory, Bishoftu, Ethiopia, for confirmation by PCR.

Molecular analysis for the detection of *Salmonella*

DNA was extracted from an overnight pure culture of each bacterial isolate during its log phase of growth. DNA extraction was carried out by using a commercially available DNA extraction and purification MINI kit (QIAGEN™, Germany) according to the manufacturer's instructions.

Conventional PCR was conducted at the National Veterinary Institute (NVI) to confirm identification made by phenotypic tests. *Salmonella* spp isolates were detected by amplifying the 496-bp segment of the histidine transport operon gene according to the methods described by Cohen *et al*. (1993). Two different primer pairs were used for this purpose (Table 1). The amplification reaction was carried out in a final volume of 22 µl comprising 3 µl nuclease-free water, 2 µl from each primer pair, 10 µl IQTM super mix (Thermo Scientific, USA), and 5 µl template. The PCR reaction conditions were 5 minutes at 94 °C for the initial denaturation, 30 seconds at 94 °C for subsequent denaturation, 30

seconds at 56 °C for the annealing, and 45 seconds at 72 °C for the elongation, and 5 minutes at 72 °C for final elongation test which is followed by holding at 4 °C until the machine is off. The total number of cycles was 32. PCR products were subjected to gel (2% agarose, USA) electrophoresis, run in TAE Buffer by loading it with ten µl of the PCR product mixed with 4µl gel red nucleic acid stain loading dye (Gelred 10000x lot 15G1214; Biotium, USA) in water and 10 µl markers (Ladder). A parallel lane was loaded with a 100 bp DNA-marker ladder (Fermantas, USA) along with known positive control *Salmonella* typhimurium (ATCC 14028) and negative control, and the products were separated at 120 V for 1 hr, then visualized under UV light and recorded using a gel documentation system (UVtec 08100554, France).

Table 1. Forward (F) and Reverse (R) primers sequences for Histidine transport operon in *Salmonella* detection.

Primer	Sequence	Target	Amplicon Size	Reference
F	5-ACTGGCGTTATCCCTTTCTCTGGTG-3'	Histidine Transport Operon	496 bp	Cohen *et al.* (1993)
R	5'ATGTTGTCCTGCCCCTGGTAAGAGA-3'			

Data management and analysis

A Microsoft Excel spreadsheet was used to enter the data. Data consistency and completeness were achieved during data collection, data entry, and analysis. The data were analyzed using a statistical package for the social sciences (SPSS), version 25. During the calculation of the animal-level prevalence of *Salmonella*, an individual animal was considered positive for *Salmonella* if at least one fecal, swab, or tissue sample was found positive for *Salmonella* as confirmed by PCR test. The proportion of *Salmonella*-positive samples was calculated by dividing the number of positive (confirmed) samples by the total number of samples investigated (processed).

Ethical consideration

Ethical approval was obtained from the University of Gondar, College of Natural and Computational Sciences, ref CNCS/02/03/189/01/2024.

Results

Cultural and biochemical detection rates of *Salmonella*

Cultural, biochemical, and molecular testing methods were used in the evaluation procedures. A PCR test was used to confirm 24 isolates that were initially suspected of being positive for *Salmonella* based on biochemical tests. However, only 11 of these isolates were confirmed to be *Salmonella* by PCR. Overall, 10 out of 120 animals tested positive for *Salmonella*, representing 8.33% prevalence (Table 2), with a 95% confidence interval ranging from 4.07% to 14.79%.

Depending on the towns where the samples and data were collected, the majority of *Salmonella*-positive isolates were found in the Kombolcha town abattoir, followed by the Gondar town abattoir (Table 2).

Table 2. Occurrence of *Salmonella* (confirmed by PCR) in cattle slaughtered in selected town abattoirs in Amhara Region (no. of animals = 120).

Towns	No. of animals sampled	No positive for *Salmonella*	Prevalence (%)
Gondar	20	3	2.5
Bahir Dar	20	0	-
Debre Markos	20	1	0.83
Debre Tabor	20	1	0.83
Kombolcha	20	4	3.33
Debre Birhan	20	1	0.83
Total	120	10	8.33

At the sample level, 11 of 480 (2.29%) samples collected from slaughter cattle tested positive, with a 95% confidence interval (CI) of 1.15% to 4.06%. Table 3 indicates the sample type and number of samples identified as *Salmonella* positive.

Table 3. Proportions of *Salmonella*-positive samples (confirmed by PCR) collected from slaughtered cattle in selected town abattoirs of Amhara Region (no. of animals = 120).

Sample type	No. examined	No. positive	% positive
Liver	120	0	-
Feces	120	4	0.83
Carcass swabs	120	2	0.42
Mesenteric lymph nodes	120	5	1.04
Total	480	11	2.29

The culture and biochemical test results of the water samples and swab samples taken from butchers' hands, coats/aprons, boots, and materials used in the slaughtering processes were all negative for *Salmonella*.

Molecular identification of *Salmonella*

The PCR amplifications gave products of 496 bp for 11 isolates by gel electrophoresis (Figure 1), which consequently confirmed them as *Salmonella*.

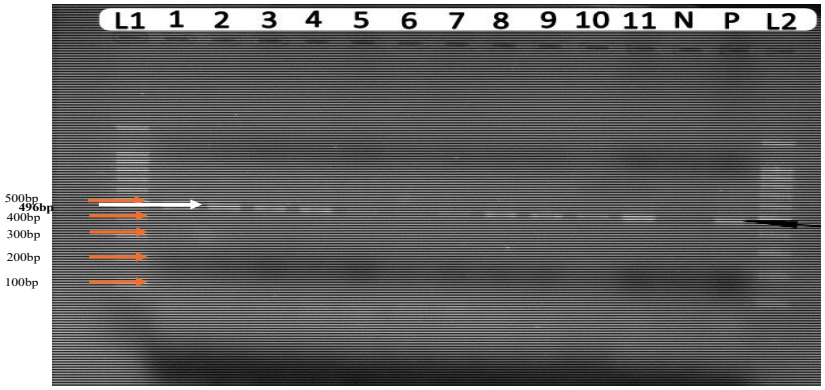

Figure 1. PCR assay to amplify Histidine Transport Operon gene of *Salmonella* isolates recovered from carcasses and feces of cattle from selected abattoirs.

Lanes 1 and 2 are ladders or molecular markers.
Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 are positive isolates for *Salmonella*.
Lane P is positive control and
Lane N is the negative control

Discussion

Our study revealed an 8.33% (10 out of 120) prevalence of *Salmonella* at the animal level. The result is comparable with previous studies in the region. Studies by Alemu and Zewde (2012), and Muluneh and Kibret (2015) reported prevalence of 7% and 7.6%, respectively, in Bahir Dar.

Our study showed a comparatively low level of contamination at the sample level. Of the 480 carcass swabs, liver, intestinal lymph nodes, and fecal samples collected, only 11 (2.29%) were found to contain *Salmonella*. *Salmonella* was found in all types of cattle samples, except the liver.

Our findings are comparable with studies that were conducted in abattoirs, such as in South Africa (2.75%) (Madoroba *et al.,* 2016), Haramaya (2.75%) (Mengistu *et al.,* 2017), Nigeria (2.6%) (Shaibu *et al.,* 2021), Iraq (2.75%) (Yousif, 2013), Addis Ababa (3.7%) (Ketema *et al.,* 2018), and Hawasa (2.0%) (Kore *et al.,* 2017). However, other researchers, including Kebede et al. (2016) (Addis Ababa), Gebremedhin *et al.* (2021) (Holeta), Wabeto *et al.* (2017) (Sodo), Beyene and Yibeltie (2015) (Asella), Alemu and Zewde (2012) (Bahir Dar), Alemayehu *et al.* (2003) (Addis Ababa), Sibhat *et al.* (2011) (Addis Ababa), Muluneh and Kibret (2015) (Bahir Dar), and Takele *et al.* (2018) (Jimma), reported prevalences significantly higher than that of the current finding. These studies reported prevalence of Salmonella from slaughtered cattle, ranging from 5.7% to 12.5%.

Although there have been several investigations on Salmonella contamination in apparently healthy slaughtered cattle, the results have not always been uniform. In this regard, the authors evaluated many papers and found that the results vary from 2.0% in Hawasa (Kore *et al.,* 2017) to 12.5% in Sodo (Wabeto *et al.,* 2017), Ethiopia. The differences in findings may be attributed to differences in the number of abattoirs included in the study, abattoir facilities, methodological differences such as study design, study period, sample size, type of sample taken, demography, area covered, diagnostic, sampling and culturing techniques, and the level of hygienic standards maintained by the abattoirs, as well as related factors that may affect the results.

In this study, compared with the other samples (liver 0 %, feces 0.83%, carcass 0.42%), mesenteric lymph nodes were identified as the most contaminated organ (1.04%). Mesenteric lymph nodes (MLN) drain the small and large in-

testine parts (jejunum, ileum, and ceacum) (Houston *et al*., 2016). The gastrointestinal tract (GIT) remains the main route of transmission for foodborne pathogens. *Salmonella* isolation from mesenteric lymph nodes indicates that the animal is naturally infected and is a carrier of the organism. Long traveling, overcrowding, and holding animals at a specific place for an extended period without feed and water can stress animals, promote *Salmonella* infection, and cause it to spread quickly around the jejuno-ileco-ceacal area, which causes swelling of the mesenteric lymph nodes (Garrido *et al*., 2014). Stress makes the animal shed *Salmonella* through feces, leading to the contamination of carcass, equipment, and abattoir environment, especially in conditions of poor hygiene.

The occurrence of *Salmonella* in MLNs in this study is comparable with the report of 1.3% from Hawassa (Kore *et al*., 2017), and 2.4% from Addis Ababa (Molla *et al*., 2003), but slightly lower than other studies, such as 3% in Iraq (Yousif, 2013), 3.2% in Bahir Dar (Alemu and Zewde, 2012), and 4.6% in Addis Ababa (Alemayehu *et al*., 2003), and still much lower than 8% (Sibhat *et al*., 2011) reported in Addis Ababa.

Salmonella isolation from feces (0.83%) reveals the pathogen's presence in the animals brought for slaughter to the abattoir. Fecal excretion is not necessarily indicative of an actual infection of the animal. After being ingested, *Salmonella* can circulate passively through the animal's gut lumen without invading the enterocytes. *Salmonella* must cross the enterocyte barrier and reach the local lymphoid system in order to cause an active infection. Carrier animals can become active fecal shedders of *Salmonella* due to the stress associated with transport and lairage, which may contribute to the spread of the infection to other animals (EFSA, 2021). The ingestion of contaminated feed, water, and pasture, as well as contaminated soil, drainages, toilet sewage, and other wastes, are some potential sources of infection. Despite the variations in the prevalence, *Salmonella* is frequently isolated from feces in various studies.

The proportion of animals found shedding *Salmonella* through their feces is different from what was recorded in other studies in Ethiopia: 4.1% (Ketema *et al*., 2018), 3.1% (Alemayehu *et al*., 2003), 6% (Sibhat *et al*., 2011), and 1.9% (Molla *et al*., 2003), all from Addis Ababa.

Two positive isolates were found from carcass swab samples. Freshly slaughtered animals' muscles are usually sterile (Kadim, 2007). Only two carcass

swab samples were found positive for *Salmonella*. Though we observed a very low prevalence of *Salmonella* (0.42%) in this study, the presence of even small numbers of *Salmonella* in carcasses and edible organs could lead to contamination of red meat and other meat products. The positive result might be due to cross-contamination from other organs due to insufficient sanitary conditions when slaughtering. Meat can be contaminated in the process of evisceration, whereby feces of contaminated animals have been transferred to the hands of the slaughtermen, which in turn contaminates other healthy carcasses. Our result is different and lower when compared with other studies, which reported a 2% (Sibhat *et al*., 2011), 2.8% (Alemayehu *et al*., 2003), and 2.5% (Ketema *et al*., 2018) carcass contamination from Addis Ababa. Other studies reported even higher values: 4.8% in Bahir Dar (Alemu and Zewde, 2012), 7.6% in Ireland (Mcevoy *et al*., 2003), and 9.8% in Addis Ababa (Nyeleti *et al*., 2000).

No *Salmonella* was isolated from the liver in this study. The finding is in accordance with the results of Alemayehu *et al*. (2003). The negative result may partly be due to the organ being far and deep situated from contamination.

In general, the level of contamination of cattle meat with *Salmonella* varies in different nations of the world. There is variation in some factors and similarity in others. Nowadays, most abattoirs have improved their hygienic conditions. The improvement is apparent in the slaughterhouses we chose for this study. We observed that they use clean and high-pressure tap water to clean the floor, tables, walls, carts, hanging chains, and hooks in the slaughterhouses. They often wash knives, axes, and other tools used for slaughtering purposes in hot water.

Isolation of *Salmonella* from various animal tissues or feces shows the spread of the organism in food animals. This emphasizes the need for regular surveillance and monitoring programs for zoonotic salmonellosis and other foodborne illnesses. It is essential to stop the organism from infecting people and spreading through tainted meat and animal products.

Contamination is likely to be worsening as meat goes through multiple chains from the time of the animal's slaughter until it reaches the customers. Meat in Ethiopia is traditionally sold in open-air retail shops without the correct hygienic display, temperature, and storage conditions. The widespread consumption of raw meat, particularly in the forms of kitfo (minced meat) and kurt (pieces of cut meat) in the study locations and across the nation, makes the

finding of *Salmonella* on beef carcasses even more significant and is believed to be another potential contributing factor to the disease's persistent presence in the community (Geresu and Desta, 2021).

Only the genus level of the bacteria (*Salmonella*) was determined in this investigation. The specific *Salmonella* serotypes circulating in the selected study abattoirs were not identified. It was also not known exactly where the contamination of the carcasses might have originated. The antimicrobial resistance pattern of isolated *Salmonella* species was not also determined. These are among some of the gaps and limitations of this study that need to be considered in future research efforts.

Conclusions

Bovine salmonellosis is a disease of significant importance to the cattle industry and public health. Consequently, improving communities' knowledge about the cause of infection and its primary sources may help lower the risk of infections in humans and cattle. The study indicates that more attention should be given to implementing and maintaining strict hygienic measurements in abattoirs before, during, and after slaughtering. As the consumption of raw and undercooked meat is a common habit in most parts of Ethiopia, public education and continuous awareness are needed to discourage the habit and minimize the occurrence of *Salmonella* infection.

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