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Short Communication

Prevalence of Salmonella and *Escherichia coli* and Associated Risk Factors Among Camel and Bovine Meat Slaughtered at Jigjiga Municipal Abattoir, Somali Regional State, Ethiopia

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Abstract: A cross-sectional study involving microbiological analysis was conducted from March 2021 to August 2021 in Jigjiga town to isolating and identifying Salmonella and *E. coli* from raw meats of camels and bovines slaughtered at the Jigjiga municipal abattoir and assessing possible associated related risk factors for the isolates. A total of 384 examined samples for the presence of *Salmonella* and *E. coli*. From the total samples examined, 64 (16.7%) and 44 (11.5%. were found to be *E. coli* and *Salmonella* positive respectively. Out of the 199 meat samples taken from camel, 31 (15.6%) and 32 (16.1%) were found positive for *Salmonella* and *E. coli* respectively while a total 185 meat samples of bovines, 13 (7%) and 32 (17%) were found positive for *Salmonella* and *E. coli*, respectively. In the univariable logistic regression analysis result, *Salmonella* revealed a statistically significant difference among different ages (OR (CI) = 2.36 (1.195–4.679); p-value = 0.013). The multivariable regression analysis showed there was no statistically significant difference between the two sex groups (OR (CI) = 2.01 (0.892–4.544); p = 0.092). In an univariable logistic regression analysis result, the odds of meat contamination in young-aged animals with *E. coli* were three times higher than in adult animals, showing a statistically significant difference (OR = 2.83 (1.567–5.095; p = 0.001). The prevalence of *E. coli* was higher in animals with poor body condition (31.9%), followed by medium (15.5%) and good body-conditioned animals (1.8%). In the multivariable logistic regression analysis, the odds of contamination of samples with *E. coli* from poor-body condition animals were 22 times higher than samples taken from good-body condition animals (OR (CI) = 21.8 (5.022 - 95.059); p = 0.000). To prevent cross-contamination of *Salmonella* and *E. coli*, hygiene must be improved, standardized procedures, and training programs should be implemented, further studies on molecular characterization and serotyping of these species are also needed.

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INTRODUCTION

The country of Ethiopia is home to the largest number of livestock owners in Africa—roughly 59.5 million cattle, 30.7 million sheep, and 30.2 million goats—but the economic benefits of these animals are negligible in comparison to their enormous numbers (CSA 2012). Almost 25% of the population are at high risk of gaining illnesses due to the significant rise in foodborne infection cases over the past 25 years (Oladunjoye and Awani-Aguma, 2023). It is well established that pathogen-contaminated meat poses a serious risk to public health (Mazi et al., 2023).

The World Health Organization (WHO 2010) estimates that foodborne and waterborne diarrheal diseases combined kill around 2.2 million people annually worldwide, the demands for protein-rich food are increasing proportionally with the increase in world population, as the main animal products like milk, meat, and eggs; are the common protein-source foods, they play an important role in global food security, nutritional well-being, and health. However, the rapid growth in livestock production and supply chains is creating public health threats associated with an zoonotic pathogen shift, which implies pandemic risks, food safety hazards, depending on the agro-ecological and socio-economic development context (FAO 2013).

The types of microorganisms and level of contamination in the dietary end-products are determined by several factors, including sanitary practices and protocols, the use of food safety interventions, the kind and degree of product handling and processing, and distribution and storage circumstances (Mendonca et al., 2020). *Listeria monocytogenes*, *E. coli O157:H7*, *Campylobacter*, and *Salmonella* species are the most common pathogens that have been linked to meat and meat products on a regular basis. Several human health cases have been connected to these organisms (Habib et al., 2021).

One of the most popular foods from animal sources is meat. For most people, it's a key component of a balanced diet because of its high nutritional value and combination of essential macro- and micronutrients. However, meat can also serve as a favourable environment for the growth of certain microbes. Salmonellosis is one of the leading food-borne illnesses from animal origin that causes bacterial gastroenteritis in humans (Ayuti et al., 2024). The two most significant foodborne pathogens of the Enterobacteriaceae family and the culprits behind foodborne illnesses spread through meat are *Salmonella spp*. and *Escherichia coli (E. coli)*, which have a negative impact on people's health worldwide (Roch et al., 2024).
Annually, millions of cases of foodborne sickness are attributed to salmonella, the second most prevalent cause of foodborne illness. Handling raw corpses and their by-products or

consuming undercooked meat can expose humans to *Salmonella* leading to in food poising (Salamah et al., 2024). Warm-blooded animals and the human digestive tract naturally contain *E. coli*, which is utilized as an indicator bacterium since it develops antibiotic resistance more quickly than other types of bacteria (Grudlewska-Buda et al., 2023).

From a sanitary perspective, the evacuation of the intestines and avoiding fecal material cross-contamination are given careful consideration. Meat has a higher incidence of E. coli and Salmonella spp. than intestinal contents. This suggests that intestinal contents are not the only sources of *Salmonella spp.* and *E. coli* contamination in meat; equipment surfaces, animal microbiota, and the environment surrounding the abattoir can also be contaminated. Bacterial contamination of water, animal microbiota, and equipment surfaces may occur during slaughtering. The environment of an abattoir can taint carcasses (Shedleur-Bourguignon et al., 2023).

Like in other underdeveloped nations, Ethiopia lacks coordinated epidemiological surveillance systems and the scope of studies makes it challenging to assess the burden of food-borne illnesses. Salmonellosis and other food-borne illnesses may have received less attention due to underreporting of cases and the existence of other more serious illnesses (Kebede *et al.*, 2016). The status of the *Salmonella* and *E. coli* from Jigjiga municipal abattoir has not been reported, however, there is limited understanding of the isolation and identification of pathogens from camel and bovine meat in Ethiopia. (Tadesse and Tessema, 2014, Kebede *et al.*, 2016) from the country's central region and export abattoirs. Therefore, the objectives of the study were to isolate and identify Salmonella and E. coli from camel and bovine meat samples collected from the Jigjiga municipal abattoir in eastern Ethiopia and to assess the possible associated risk factors contributing to the contamination of meat with the isolates.

MATERIALS AND METHODS

Ethical approval

This research was approved by the Research Ethics Review Committee of College of Veterinary Medicine, Jigjiga University. The study was conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All methods were carried out in accordance with relevant guidelines and regulations. Before conducting the study, the objectives, expected results, and benefits of the study were explained to the abattoir workers who participated in the study and written informed consent was obtained from all participants.

Description of Study Area

Jigjiga is a city in eastern Ethiopia and the capital of the Somali Regional State. Based on figures from the Central Statistical Agency, Jigjiga has an estimated population of 98,076 people, of whom 50,355 are males and 47,721 are females. There is one substandard abattoir in Jigjiga city, owned by Jigjiga municipality and the environmental protection office, that aims to provide officially inspected and safe meat (beef, camel, goat, and mutton) for consumers. The abattoir has separate compartments for animals' slaughter based on Christian and Islamic faiths. On average, 41 cattle for Christians and 20 for Muslims are slaughtered per day, and an average of 15 camels are slaughtered per day. The numbers of slaughtered animals are much higher than the capacity of the abattoir. In addition, the city has ten l camel butcher shops (CSA, 2018).

Study Population

The study populations were apparently healthy bovines and camels slaughtered at the Jigjiga municipal abattoir, in respective to the animal age, sex, breed, body condition score, and origin. A cross-sectional study involving microbiological analysis was employed and conducted from March to August 2021 in Jigjiga, Ethiopia.

The formula in Thrusfield (2018). was used to calculate the sample size using basic random sampling techniques and a 95% confidence interval with 5% precision.

$$N = \frac{1.96^2 Pexp\ (1- Pexp)}{d^2}$$

Where; n required sample size
P = expected prevalence
D = required precision

The expected prevalence of contaminated meat by Salmonella and E. coli SPP estimated to be 50% with the required precision (d) of 5% (0.05). The sample size was determined by calculating the value in the given formula.

$$n = \frac{1.96^2 x\ 0.5(1-0.5)}{(0.05)^2}$$

$$= \underline{384}$$

Sampling Technique and Sample Collection Producers

Duplicate carcass samples from different portions were collected by a systematic random sampling technique. Swabs and meat samples were taken according to the method described in ISO-17604 (2003). The abdomen (flank), thorax (lateral), crutch, and breast (lateral) were the sampling sites. The sampling areas were delineated by using a 10 x 10 cm aluminum foil template. A sterile cotton-tipped swab (2 x 3 cm) fitted with the shaft was first soaked in approximately 10 ml of buffered peptone water (BPW) and rubbed over the delineated area horizontally and then vertically several times. The cotton swab was left in the universal bottle after the rubbing process was finished and the swab was disposed of by dipping it into the buffered peptone water and breaking off the wooden shaft that was pressing on the inside. The other designated regions were swabbed with additional identical swabs and put into the same container. As before, a second dry, sterile cotton swab of the same kind was used over the whole sampled area as mentioned above, and it was put into the same container. Meat samples weighing twenty-five grammes were collected, preserved in sterile plastic bags, and refrigerated in an ice box with ice packs. The sampling template (mostly aluminum foil templates (10 × 10 cm)) was removed from its bag and placed over the desired sampling site on the meat carcass. Samples were then homogenized using a meat grinder under aseptic conditions and stored for further analysis. Finally, it was then immediately transported to the microbiology laboratory of the Jigjiga Regional Veterinary Diagnostic and Research Centre in a cooler with ice packs for examination. Samples were collected within 12 hours post-slaughter and during mid-day around 7:00 p.m. local time to minimize the microbial changes due to environmental temperatures and post-slaughter timings

Isolation and Identification of Salmonella

Salmonella was isolated and identified as recommended by the International Organization for Standardization (ISO-6579, 2002). The bacteriological media were prepared according to the manufacturer's instructions.

For isolation of *Salmonella*, samples were directly inoculated into nutrient agar for general enrichment. The nutrient media was incubated overnight at 37˚C under aerobic conditions. Following incubation.

A loop-full of each culture was streaked onto the surface of *Salmonella Shigella* agar, and XLD (xylose lysine deoxycholate) and incubated for 24 hours at 37˚C in an aerobic environment. Two colonies were selected from each plate, based on their appearance and selected colonies were examined for morphology using Gram´s stain, Hydrogen sulphide (H2S) Production, Indole Test, Motility test, and Catalase Test.

The samples were streaked onto nutrient agar and cultured for 24 hours at 37 ºC to isolate *E. coli.* The samples were subculture onto EMB and MacConkey (MAC) agar the following day, and they were incubated for 24 hours at 37˚C under aerobic conditions. Three colonies from each sample that met the criteria for lactose fermentation and pink coloration on MAC agar—a selective medium for Enterobacteriaceae organisms—were tested biochemically. *E. coli* was identified based on standard tests, such as IMViC, MIO, and TSI.

Statistical analysis

The collected data was entered into EPI Data version 3.1 and then exported to SPSS version 20 for analysis. To characterize the prevalence of bacterial isolates, descriptive statistics were employed, with results expressed as percentages. Chi-square tests were utilized to evaluate any statistically significant differences in *Salmonella* and *E. coli* contamination between camel and bovine meat samples. Additionally, to assess potential risk factors associated with *Salmonella* and *E. coli* contamination, univariable logistic regression analysis was employed. This analysis examines the association between individual risk factors (e.g., animal age, body condition score) and the presence or absence of bacterial contamination. Variables with statistically significant associations (p-value < 0.05) in the univariable analysis were then included in a multivariable logistic regression model. This model helps identify independent risk factors while controlling for the effects of other variables. Throughout the analysis, a p-value of less than 0.05 was considered statistically significant.

RESULT

Overall Prevalence of *Salmonella* and *E. coli*

From a total of 384 carcass meat samples examined for the presence of *Salmonella* and *E. coli*, 64 were found to be E. *coli* positive with an overall prevalence of 16.7%, and 44 were found *Salmonella* positive showing an overall prevalence of 11.5% (Table 1). Out of the 199 meat samples taken from camel, 31 (15.6%) and 32 (16.1%) were found positive for *Salmonella* and *E. coli* respectively. From the total 185 meat samples of bovines, 13 (7%) and 32 (17%) were found positive for *Salmonella* and *E. coli*, respectively (Table 2,3).

Table 1: An overall summary of *Salmonella* and *E. coli* isolates from raw meat samples obtained from Jigjiga municipal abattoir.

Isolates	Total Sample Examined	Sample Positive	Prevalence (CI)	P-value
Salmonella	384	44	11.5 (8.2-14.6)	0.886
E. coli	384	64	16.7 (13.0-20.4)	

Prevalence of *Salmonella* about Various Variables

In the study area, Fiiq (15.1%) and Jarar (13.9%) were found with slightly higher prevalence, followed by Fafan (9.6%) and Nogob (10.0%). However, there was no statistically significant difference in the occurrence of *Salmonella* among the different origins (p > 0.05). The sex groups of the animals examined for the prevalence of *Salmonella* revealed 12.5% and 10.6% in female and male animals, respectively. The univariable logistic regression analysis result revealed a higher rate of meat contamination with *Salmonella* in young-aged animals (19.7%) than in adults (9.4%), with a statistically significant difference (OR(CI) = 2.36 (1.195–4.679); p-value = 0.013). The multivariable regression analysis showed there was no statistically significant difference between the two sex groups (OR (CI) = 2.01 (0.892–4.544); p = 0.092). In this study, the prevalence of *Salmonella* was higher in animals with poor body condition (22.7%), followed by medium (7.7%) and good body-conditioned animals (4.5%), and the variation was statistically significant (P<0.05). This result shows that the meat contamination with *Salmonella* is higher in camels (15.6%) than bovines (7.0%), with a statistically significant difference (P<0.05) (Table 2).

Prevalence of *E. coli* about Various Variables

a slightly higher prevalence of *E. coli* was obtained from Fafan (19.3%) and Jarar (18.0%), followed by Nogob (14.3%) and Fiiq (10.6%), with no statistically significant difference (p > 0.05). This result shows that the meat contamination with *E. coli* is higher in bovine (16.1%) than in camel (17.3%) with no statistically significant variation (OR = 0.92; CI = 0.535–1.567; p = 0.395). In a univariable logistic regression analysis result, the odds of meat contamination in young-aged animals (30.3%) with *E. coli* were three times higher than in adult animals (13.3%), showing a statistically significant difference (OR = 2.83 (1.567–5.095; p = 0.001). Our findings showed that the prevalence of *E. coli* was higher in animals with poor body conditions (31.9%), followed by medium (15.5%) and good body-conditioned animals (1.8%). In the multivariable logistic regression analysis, the odds of contamination of samples with *E. coli* from poor-body condition animals were 22 times higher than samples taken from good-body condition animals (OR (CI) = 21.8 (5.022 - 95.059); p = 0.000) (Table 3).

Table 2: Prevalence of *Salmonella* isolated from meat samples of camel and bovine slaughtered at Jigjiga municipal abattoir about various risk factors

Variable	Categories	No. examined	No. positive	Prevalence	Univariable logistic regression analysis		Multivariable logistic regression analysis	
					OR (95% CI)	P-value	OR (95% CI)	P-value
Origin	Nogob	70	7	10	Ref.		Ref.	
	Fafan	176	17	9.7	0.96 (0.380-2.432)	0.935	1.81 (0.619-5.305)	0.277
	Fiiq	66	10	15.2	1.6 (0.573-4.505)	0.367	1.98 (0.653-6.010)	0.227
	Jarar	72	10	13.9	1.45 (0.519-4.056)	0.477	1.95 (0.649- 5.870)	0.233
Age	Adult	308	29	9.4	Ref.		Ref.	
	Young	76	15	19.7	2.36 (1.195-4.679)	0.013	3.80 (1.475-9.827)	0.006
Sex	Male	244	26	10.6	Ref.		Ref.	
	Female	140	18	12.5	1.23 (0.651-2.347)	0.15	2.01 (0.892-4.544)	0.092
Body Condition	Good	110	5	4.5	Ref.		Ref.	
	Medium	155	12	7.7	1.76 (0.602-5.154)	0.301	1.11 (0.357-3.475)	0.851
	Poor	119	27	22.7	6.16 (2.279-6.659)	0.000	4.31 (1.510-12.313)	0.006
Species	Bovine	185	13	7.0	Ref.		Ref.	
	Camel	199	31	15.6	2.44 (1.234-4.826)	0.010	4.4 (1.793-10.786)	0.001

OR: Odds ratio; CI=confidence interval

Table 3: Prevalence of *E. coli* isolated from meat samples of camel and bovine slaughtered at Jigjiga municipal abattoir about various risk factors

Variable	Categories	No. examined	No. positive	Prevalence	Univariable logistic regression analysis		Multivariable logistic regression analysis	
					OR (95% CI)	P-value	OR (95% CI)	P-value
Origin	Nogob	70	10	14.3	Ref.		Ref.	
	Fafan	176	34	19.3	1.43 (0.667-3.093)	0.355	1.74 (0.688-4.380)	0.242
	Fiiq	66	7	10.6	0.71 (0.253-1.995)	0.518	0.72 (0.243-2.136)	0.555
	Jarar	72	13	18.0	1.32 (0.537-3.249)	0.543	1.44 (0.541-3.833)	0.465
Age	Adult	308	41	13.3	Ref.		Ref.	
	Young	76	23	30.3	2.83 (1.567-5.095)	0.001	2.13 (0.999-4.556)	0.050
Sex	Male	244	40	16.4	Ref.		Ref.	
	Female	140	24	17.1	1.1 (0.605-1.838)	0.850	1.38 (0.694-2.773)	0.354
Body Condition	Good	110	2	1.8	Ref.		Ref.	
	Medium	155	24	15.5	8.89 (2.286-42.805)	0.002	1.12 (2.070-40.191)	0.003
	Poor	119	38	31.9	25.3 (5.937-108.085)	0.000	21.8 (5.022-95.059)	0.000
Species	Bovine	185	32	17.3	Ref.		Ref.	
	Camel	199	32	16.1	0.92 (0.535-1.567)	0.749	1.35 (0.672-2.725)	0.395

OR: Odds ratio; CI=confidence interval

DISCUSSION

The prevalence of *Salmonella* in carcasses was estimated at 11.4%. Some previous studies in Ethiopia show a slightly lower result than ours. For instance, Akafete and Haileleul, (2011) and Tadesse and Gebremedhin (2015) found an estimated *Salmonella* prevalence of 8.3% at Modjo and 8.6% at central, eastern, northern, and southern Ethiopia, respectively, from carcass samples. This prevalence difference could be explained because of differences in the hygienic and sanitary practices practiced in the abattoirs. In the current study of the municipal

abattoir, poor sanitation and hygienic standards have been practiced in comparison to the export abattoirs.

As observed during sampling, there is a shortage of disinfectants, hot water, and a separate room for the final carcass and live animals in the abattoir, which contributes to the to the contamination of the carcass with the pathogens. The overall high level of carcass contamination with *Salmonella* and *E. coli* is a special public health hazard for a country like Ethiopia, where raw and undercooked meat is the favourable in most areas (Akafete and Haileleul, 2011).

Salmonella can live and grow in the rumen of malnourished animals, as is widely known. Moreover, healthy carriers rarely excrete more than a few *Salmonella* unless they experience stress, such travelling (Libera et al., 2022). Animals feces are a source of contamination, high levels of *Salmonella meat* contamination may be linked to excessive growth of the bacteria as a result of exposure to predisposing conditions like malnutrition, crowded markets, and transportation, which in turn increase exposure to other animal feces. Given that most people in Ethiopia prefer to eat raw or undercooked meat, the high incidence of *Salmonella* contamination in carcasses is particularly concerning for public health (Akafete and Haileleul, 2011).

Table 1 indicates that *E. coli* 64 (16.7%) was more common in the carcass samples than *Salmonella* 44 (11.4%). This result contradicts certain prevuosly published research. For instance, Akbar et al., (2014) reported that *E. coli*. isolated from 25% of poultry meat in Thailand, while (Yulistiani et al., 2007) reported that *E. coli* was identified in 77.5% of chicken flesh in Indonesia. Variations in the environment, research seasons, personnel' understanding of contamination techniques, and animal management in the lairage could all be contributing factors to the disparities in the incidence of *E. coli* throughout studies.

The increased prevalence of young animals with ≤6 years of age in both *E. coli* (30.3%) and *Salmonella* (19.7%) compared to the rest of the of the age groups could probably reflect lowered body defenses in young animals. Furthermore, the close interaction of the sucklers with infested lactating females could also be another factor that makes them more liable to the disease, leading to a higher prevalence in this age group (Köllmann et al., 2021)

On the other hand, the higher prevalence of female animals than that of male animals in both *E. coli* (17.1%) and *Salmonella* (12.5%) in the study might be due to hormonal influences, i.e., the higher levels of prolactin and progesterone hormones could make the females more susceptible to any infection. Additionally, pregnancy and lactation stress could also aggravate the susceptibility of the female camels to infections (Khalphallah et al., 2024).

CONCLUSIONS

The present study indicated detection of Salmonella and E. coli with an overall prevalence of 7.0% and 17.3% from meat samples of healthy slaughtered bovine and 15.6% and 16.1% prevalence from camel meat samples collected at Jigjiga municipal abattoir, respectively. The results of the present study indicate poor evisceration processes and hygienic practices of the workers, which could result in the contamination of carcasses and cross-contamination from positive animals. Meat samples obtained from young and poor-body-conditioning animals showed a significantly higher proportion of isolates in both cases. Food safety educational programmes in slaughterhouses and markets and public education as regards the risk of consumption of raw and uncooked animal products are important lines of defense against Salmonella and E. coli. Further studies are needed to investigate the relationship between Salmonella and E. coli and public health in the area.

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Conflicts of Interest: The authors declare no conflicts of interest.

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