Occurrence and Antimicrobial Susceptibility of *Salmonella* in Fecal and Carcass Swab Samples of Small Ruminants at Addis Ababa Livestock Market

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Abstract

A cross sectional study was undertaken to investigate the occurrence and antimicrobial susceptibility of *Salmonella* from small ruminants brought for sale at different markets of Addis Ababa and those slaughtered at abattoir. Different sample types (fecal, carcass swab, meat and feed) were collected and cultured for *Salmonella* using standard procedure by pre-enriching in Buffered Peptone Water and enriching in Rappaport- Vassiliadis enrichment Broth (RVB) and Tetrathionate broth (TTB). It was then streaked from both RV and TTB to Xylose Lysine Deoxycholate (XLD). Presumptive Salmonella colonies were confirmed by various biochemical tests and *Salmonella* Genus Specific Polymerase Chain Reaction (PCR). Salmonella isolates were tested for their susceptibility to 17 antimicrobials. The overall occurrence of *Salmonella* in sheep and goat fecal samples at Addis Ababa livestock market was 4.08% (12/294) (95% CI: 1.8- 6.4%) and its occurrence in carcass swab samples collected from Addis Ababa public abattoir was 0.85% (1/117) (95% CI: 0.8-2.5%). There was no statistically significant difference in occurrence of *Salmonella* in sheep and goats (p>0.05). But nearly a significant difference (p=0.052) for the occurrence of *Salmonella* between the carcass swab of goats (3.12%) and sheep (0.0%) was seen. All the 13 *Salmonella* isolates were susceptible to the four drugs (amikacin, cefoxitin, chloramphenicol and nalidixic acid) and 92.30% of the isolates were susceptibile to Ceftriaxone and Nitrofuranation. Resistance to streptomycin was observed in 84.62% of the *Salmonella* isolates followed by gentamicin (67%), tetracycline (54%) and trimethoprim (53%). Intermediate resistance was seen towards cephalothin, neomycin and ciprofloxacin in 23.08%, 15.38% and 15.38% of the isolates, respectively. All of the 13 *Salmonella* isolates were resistant to at least one of the 17 antimicrobials tested. Seven (53.85%) were resistant to 4-8 antimicrobials. In conclusion, *Salmonella* is more common in fecal samples of sheep than the carcass swab and most of the isolates were multidrug resistant. To hamper the burden of *Salmonella* infection and contamination in live animals and animal products, it is critical that risk reduction strategies should be implemented throughout the food chain.

Keywords: Salmonella sheep • Goat • Feces • Carcass • Antimicrobial

Introduction

Salmonella are facultative anaerobic Gram-negative rods within the family of *Enterobacteriaceae*. They can grow at an optimum temperature of 37C, and can be killed by temperature of 55oC [1]. It is closely related to the genus *Escherichia* and is found worldwide in cold-blooded and warm blooded animals as well as in non-living habitats [2]. Most *Salmonella* catabolize a variety of carbohydrates with the exception of *Salmonella* serotype Typhi which only produces acid, whereas lactose and sucrose are not fermented by the majority of *Salmonella* [3,4].

Salmonellosis is economically important infectious disease of animals [5]. *Salmonella* infection in animals occur mostly through the ingestion of contaminated feed and water or through contact with the contaminated excretions of latently infected animals; shedders always playing a major role in the dissemination of the organisms [6]. Though primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry [7].

Salmonella are readily transferred from animal to animal, animal to humans, and human to human by direct or indirect pathways [8]. Food borne *Salmonella* typically causes acute gastroenteritis and may cause a more septicemic disease usually in very young, the elderly and immune compromised subject *S. Infantis, S. Butantan, S.*

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Braenderup, *S. Kingabwa*, *S. Zanzibar*, *S. Kottbus*, *S. Anatum*, *S. Typhimurium* and *S. Hadar*, *S. Kottbus*, were the most common serotypes isolated from samples originated from apparently healthy slaughtered sheep and goats Addis Ababa supermarkets and Elfora Debre Zeit abattoir [9]. The use of antimicrobials for prophylaxis in food producing animals has been a great concern, because it is believed to be an important factor in the emergence of strains resistant to certain antimicrobials. However, the exact causes of the emergence of these resistant strains have yet to be pinpointed [10]. Streptomycine, chloroamphinicol, ampiciline, ciprofloxacin, gentamycine, ceforixicine, amoxaciline, kanamycine, nalidixic acid, tociprofloxacine are commonly used drug in Ethiopia. Study by Zelalem, et al. and Fluckey, et al. states use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risk associated with consumption of contaminated meat products.

Ejeta, et al. reported a 14.1% *Salmonella* contamination rate of mutton collected from Addis Ababa supermarkets, and other studies on prevalence of *Salmonella* in various food animals and animal products in Ethiopia. Even if there are different studies carried out in Addis Ababa on sheep and goats and other food animals, little information is available on prevalence and antimicrobial resistance of *Salmonella* in small ruminants at Addis Ababa market and ready for sale and slaughtering. Therefore the objectives of this study are

To determine the occurrence of *Salmonella* in feces of small ruminants brought from various regions of the country to Addis Ababa markets and also in meat and carcass swab samples collected from Addis Ababa abattoir and butcher shops.

To determine the antimicrobial resistance of isolated *Salmonella*.

Materials and Methods

The study was conducted in Addis Ababa, the capital city of Ethiopia. The city lies at 9°1'' North latitude, 38°44'' East longitude and altitude range of 2000-3000 meters above sea level/masl/. Its annual rain fall ranges from 750-1500 millimeters and the average daily temperature is about 16°C [11]. Specifically, four sheep and goats'' market (Akaki-Kality, Nock_18, Kera, Kara and Yeka) areas within Addis Ababa and Addis Ababa municipal abattoir (during antemortem examination) were used for sample collection from live animals. Abattoir and butcher shops in the respective market areas were also used for carcass swabs and meat sample collections. The laboratory work for the samples was conducted in Microbiology laboratory of Aklilu Lemma Institute of Pathobiology of Addis Ababa University. A cross-sectional study design was conducted to investigate occurrence of Salmonella in feces of small ruminants sold in Addis Ababa and carcass swab and meat samples from slaughtered small ruminants. The sample size required for the study was determined depending on the expected prevalence of salmonellosis and the desired absolute precision as per the method of Thrusfield (1995). Using a 95% confidence level, 5% absolute precision and 7.4% expected prevalence for goats and sheep (Tigist, 2017 4.17%); a sample size of 61.4 was expected. Even though, the desire sample size is as indicated here; for sake of better representative sample size 234 sheep and 60 goats were considered for fecal sample during the study period. Moreover, 117 carcass swab

samples, 10 pieces of meat samples and 9 feed samples were also sampled purposely. Study population were all sheep and goat population sold on open air markets in Addis Ababa at various places. A total of 294 randomly selected animals for fecal sample n= 234 for different sheep breeds and n=60 for goats brought from various regions of the country to Addis Ababa live stock market Akaki-Kality, Nock_18, Kara and Yeka areas and Addis Ababa abattoir individually or in flocks by vehicle to be sold and slaughtered were used as study animal. Randomly selected carcass swabs from, ramp, sternum, brisket and neck area of sheep (n=85) and goats (n=32) slaughtered in Addis Ababa abattoir by using gauze were used as additional sample. More over feed samples (n=9) from the watering and feeding troughs and piece of meat samples (n=10) from butcher shops were also used for the study.

On each sampling day, from each animal market area, once oral consent from the animal owner was obtained (species, breed, sex and body conditions were concerned), the sheep types in Ethiopia were classified into four major groups based on their physical characteristics: short fat-tailed, long fat-tailed, thin-tailed and fat-rumped sheep. Based on DNA differences, Ethiopian sheep types have been classified into nine genetically distinct breeds [12]. Scoring sheep and goats is done using a BCS ranging from 1.0 to 5.0, with 0.5 increments. according to previously described procedures [13]. An animal of BCS 1.0 is extremely thin with no fat reserves and a BCS of 5.0 is a very over-conditioned (obese) animal. In most cases, healthy sheep and goats should have a BCS of 2.0 to 3.5. A BCS below 2.0 indicates a management or health problem. A BCS of 4.5 or 5 is almost never observed under normal management conditions. To assign a BCS, one must touch and feel the animal. In sheep, the lumbar region is the principal site for BCS determination while in goats the rib cage and sternum also play a role. Before scoring the animal should be standing in a relaxed position. It should not be tense, crushed by other animals or held in a crush. If the animal is tense it is not possible to feel the short ribs and get an accurate condition score. It shows body conditions scoring guidelines used in this study.

Sheep and goats to be involved in the study were randomly selected for sampling. From each selected animal, about 10gms of feces was collected separately in to sterile zippered plastic bags by using examination glove directly from the rectum. Moreover, carcass swabs and meat samples from Addis Ababa abattoir and butcher shops and feed samples from feeding and watering trough were collected in aseptic manner as additional sample. Then each sample was appropriately labeled and accompanied by necessary identifying information, which include date of sampling, type of sample, species of animal, and identification code. Samples were then transported with ice box directly to Microbiology Laboratory, Aklilu Lemma Institute of Pathobiology, Addis Ababa University and processed for Salmonella isolation.

Results

A total of 234 sheep and 60 goats for fecal samples, 117 carcass swab samples (n=85 for sheep and n=32 for goats), 10 meat samples (n=6 for sheep and n=4 for goats) and 9 feed samples were cultured for Salmonella. As shown, the overall occurrence of Salmonella in sheep and goat fecal samples at Addis Ababa livestock market was 4.08% (12/294). Its occurrence in carcass swab samples collected

from Addis Ababa public abattoir was 0.85% (1/117). Though, relatively higher occurrence of Salmonella was found in sheep (4.7%) than goats" (1.66%) fecal samples, there was no statistically significant difference (p>0.05) between these two species. And also there was no significant difference (p>0.05) in occurrence of Salmonella between the carcass swab samples of goats (3.12%) and sheep (0.0%). Salmonella was not isolated from any of the sheep carcass swab samples (n=85) collected from Addis Ababa public abattoir.

Salmonella was identified from 13 (3.023%) samples of which 92.3% were from fecal and 7.7% were from carcass swab samples. Salmonellae was isolated from 12 (4.08%) of 294 fecal samples and only one isolate was obtained from 1 (0.85%) of 117 carcass swab samples; but no Salmonella was detected from the 10 meat and 9 feed samples. The proportion of positive samples therefore ranged from 0% in meat and feed to 4.08% in fecal.
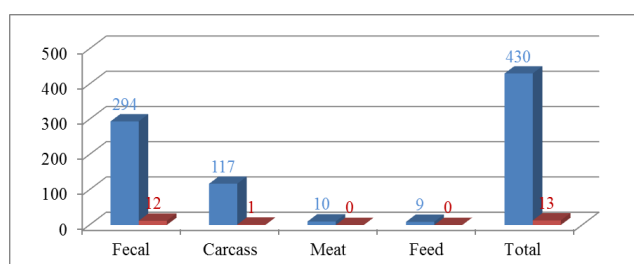

Figure 1. Sample types with their respective observations versus occurrence of Salmonella.

The embryonal and early larval development of the cardiovascular system is excellently visualized by confocal micro-angiography. This technique is well suited for optically clear (transparent) thin animals but fail in opaque and thick objects. Here SEM of VCCs can be applied. Our group focusses upon spatio-temporal aspects of growth and regression of blood vessels in the Xenopus laevis model organism. Xenopus is an anuran amphibian and undergoes drastic changes in basically every organ/tissue during metamorphosis. Most obvious is the loss of larval-specific organs, like the gills and tail. These organs are highly vascularized in early stages of metamorphosis where the growth of blood vessels dominates. At the height of metamorphosis (climax), gills and tail are resorbed and regression of a highly differentiated, complex vascular system can be studied. But also the microvascular anatomies of chick embryos, mouse embryos, rat embryos and of isolated human fetal organs have been successfully studied by this technique. Angiogenesis research is another field of application of SEM of VCCs. Here blood vessels that undergo sprouting and/or non-sprouting angiogenesis can be identified and localized (Figure 1).

A total of 12 Salmonella isolates were confirmed by boiochemical tests and PCR. Figure shows the PCR Gel image of the twelve Salmonella isolates confirmed by PCR out of the 20 tested (Figure 2).
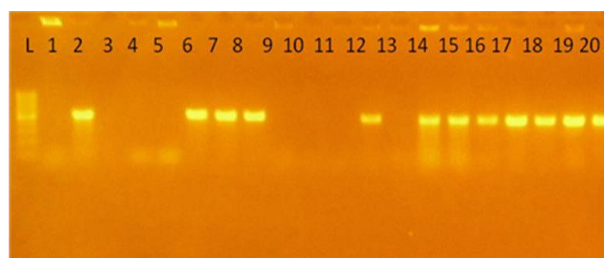

Figure 2. PCR amplification of suspected Salmonella isolates using genus specific PCR.

Discussion

Higher isolation of Salmonella was found in sheep (4.7%) than goats (1.66%) fecal samples, this higher occurrence in sheep might be due to differences in feeding behavior between sheep and goat, sheep prefer to graze while goat to browse and rearing area as well as management differences. Close contact and holding time during journey and at arrival in market area which predispose to cross contamination through poor hygiene of the market environment. Though feed samples were negative in this study which might be due to small sample size; despite the fact that, it needs further investigation to identify clearly the factors which are responsible for the variation.

Though relatively high Salmonella occurrence was observed in male and good body conditioned animals compared to females and animals with poor body condition, there was no significant difference. The observed minor difference could be attributed to the difference in sample size in both cases. The probable reason why Salmonella prevalence was low in carcass swab samples compared to fecal samples of small ruminants in the current study could be due to good hygienic practices if the abattoir workers with minimal contamination of the carcass with fecal material.

Salmonella isolation rate of 4.7% in fecal samples of sheep in this study was in agreement with different findings: 4.17% in addis ababa livestock market and abattoir enterprise by Tigist (2017), 3.3% from sheep feces in Jimma, 4.8% isolates in the fecal samples of apparently healthy slaughtered sheep in Debrezeit abattoirs and sheep and goat in central Ethiopia and 4.8% in Ethiopia, However, it was relatively higher than the 1.04% isolation rate who reported from different organs and feces of apparently healthy sheep and goats slaughtered at Addis Ababa Abattoir Enterprise and 2.1% in Ethiopia [14-16]. The 1.7% prevalence in goat feces in this study was lower than that of 3.3% in Ethiopia [17]. The occurrence of Salmonella (0.85%) in carcass sample in this study was lower than the findings of Woldemariam accounting for 6.4% at Elfora Debre Zeit abattoir in Ethiopia and Wassie which also reported 11.5% for sheep and 3% for goats. 6.19% Isolation of Non- Typhoidal Salmonella from Sheep feces in Eastern Hararghe, 7.7% from the fecal samples of sheep slaughtered in Export Abattoir of Modjo Ethiopia. The difference in the prevalence could be due to differences in study period, study site (abattoirs), study population and number of samples examined between the studies [18].

In the present study 53.85% of the Salmonella isolates from sheep and goats were multiple drug resistant to antimicrobial drugs commonly used to treat bacterial infections in domestic animals and

humans in Ethiopia. This finding supports the well known fact that antimicrobial resistance rate of Salmonella isolates in Sub-Saharan Africa is generally high which is primarily attributed to the indiscriminate use of antimicrobials both in animals and human health sectors [19].

Salmonella isolates showed substantial resistance to streptomycin, ampicillin, gentamycin, tetracycline and trimethoprime. It has been suggested that emergence of multiply-resistant Salmonellae can be the product of conjugative transfer of R-plasmids between bacterial species. Agricultural use of sub therapeutic doses of a single antibiotic could select for bacterial strains harboring plasmids with multiple resistance codons [20]. The emergence and prevalence of multiply-resistant Salmonellae in meat animals can seriously compromise public health. This might limit therapeutic choice to manage salmonellosis and other bacterial diseases. In general, antimicrobial use is a key driver of resistance development, which could be irrational use of antimicrobials due to lack of access to appropriate treatment and underuse due to inadequate dosing, poor adherence or use of substandard antimicrobial and lack of financial support to complete treatment course.

Conclusion

In this study the occurrence of Salmonella in sheep and goats was 4.08% and 0.85% respectively for the samples collected from Addis Ababa livestock markets and Addis Ababa public abattoir as well as bucher's shop of different sub-cities. Higher isolation was observed in feces of sheep and goats samples as compared to other sample types. Salmonella was not detected from feed and meat samples from butcher shops. To control and prevent Salmonella infection and contamination in live animals and animal products, it is critical that risk reduction strategies should be used throughout the food chain. Based on the findings of the present study, the followings are recommended:

Even though the rate of occurrence of Salmonella in carcass is not that much higher, ligation of the esophagus and gut during evisceration and immediate separation of the offal from the carcasses should be employed, in order to reduce contamination from gastrointestinal content.

Further investigation on characterization of the Salmonella isolates to identify the species and serotype should be carried out

Further detailed studies should be conducted to undertake molecular characterization of resistant genes associated with the observed resistance phenotype

Since antimicrobial resistance problem is expanding, appropriate national regulations and control strategies on use and application antimicrobial agents should be designed.

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