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Isolation of Non-thyphoidal *Salmonella* from Goat feaces in Haramaya District, Eastern Hararghe

Yalew Abiyu Senbeto*

Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center, P.O. Box 25, Pawe, Ethiopia

Abstract

Nontyphoidal *Salmonella* is most important food borne zoonotic pathogens causing gastro enteritis both in developed and developing countries of the world. They represent an important human and animal pathogen worldwide. A cross sectional study was conducted to isolate Nontyphoidal *salmonella* from goat feaces at Haramaya district, specifically Becheke and Ifa Bate peasant associations. A total of 126 feacal sampe was directly collected from rectum and processed in laboratory based on protocol recommended by the International Standardization Organization designed for isolation of *salmonella* (ISO-6579, 2002) with some modifications. Out of the total 126 goat faecal samples examined, 4(3.2%) was detected to be positive. The prevalence was slightly higher in Ifa Bate (4.9%) than 1(1.5%), Becheke with no significant difference in the prevalence. The prevalence of *salmonella* was also found to be higher in young goats 1(8.3%), followed by old 2(5.3%) and adult 1(1.3%) goats with no significant statistical association between the age groups. The prevalence of *salmonella* was also found to be slightly higher in female goats. Since there were no previous study in the area, this study indicate that the necessity of a further Investigation on the isolation, identification and antimicrobial susceptibility and Epidemiology of non-typhoid *salmonella* from goat feces.

Keywords: Feaces • Goat • Haramaya district • Isolation • Nontyphoidal *salmonella* • zoonotic

Introduction

The genus *salmonella* obtained its name from the American bacteriologist and veterinarian Daniel Elmer Salmon, who first isolated *salmonella enterica* serotype Cholerae suis from intestine of a pigs in 1885. It currently consists of two species that are 95- 99% homologous on a DNA level [1]. These are *salmonella enterica* and *salmonella bongori*. According to the current White-Kauffmann-Le Minor Scheme, *salmonella enterica* is further divided into six subspecies; *S. enterica* subspecies *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), *S. enterica* subsp. *indica* (VI).

There are currently over 2,600 *salmonella* serotypes, majorities (99%) of which are found in *S. enterica* and almost 60% in *S. enterica* subsp. enterica (I). Most of the serotypes pathogenic to humans and animals belong to *salmonella enterica* subsp. enterica. *Salmonella enterica* subsp subspecies *enterica* strains are usually isolated from humans and warm blooded animals, whereas *S. enterica* subsp. *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), *indica* (VI) and *S. bongori* are usually isolated from cold-blooded animals and the [1,2].

Salmonella are Gram-negative, non-spore forming, facultative anaerobic rods belonging to the family Enterobacteriaceae. They are motile (except S. Gallinarum and S. Pullorum), non-capsulated (except S. Typhi, S. Paratyphi C and some strain of S. Dublin) and non-fastidious bacteria that do not require sodium chloride for growth, but can grow in the presence of 0.4 to 4%. *Salmonella* catabolize a variety of carbohydrates into acid and gas, utilize citrate as the sole carbon source, reduce nitrates to nitrite, don't produce cytochrome oxidase, produce H2S and decarboxylate lysine, but failed to metabolize lactose, sucrose and urea. They are also oxidase negative, catalase positive, indole and Voges-Proskauer negative, and methyl red and Simmons citrate positive. Some of these characteristics are used for biochemical confirmation of *salmonella*.

Salmonella constitute serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease. *Salmonella enterica* serovar Typhi and *salmonella enterica* serovar Paratyphi A and B cause enteric fever, a systemic febrile illness, occurring only in humans that is distinguished from the more commonly self-limited acute gastroenteritis caused by the group of Nontyphoidal *Salmonella* (NTS). NTS are considered one of the most important food borne zoonotic pathogens causing gastroenteritis both in developed and developing countries of the world. They represent an important human and animal pathogen worldwide. A characteristic feature of *salmonella* is its wide host range including mammals, birds, and cold-blooded animals in addition to humans. It primarily inhabits the gastrointestinal tracts of animals [3,4].

salmonella is a leading cause of food borne illness worldwide and can cause enterocolitis (salmonellosis), enteric fever (typhoid fever), and septicemia the principal clinical syndromes being enteric fever and gastroenteritis (Miller and Pegues, 2000). Nontyphoidal salmonellosis or enterocolitis is a worldwide disease of humans and animals caused by at least 150 *salmonella* serotypes, *salmonella* Typhimurium and *salmonella* Enteritidis being the most common serotypes. These two serovars can colonize the alimentary tract of animals without causing disease so that their contamination of human food chain can be a significant health concern [2].

Enteric fever (Typhoid fever) is a protracted systemic illness that results from infection with *salmonella* Typhi and *salmonella* Paratyphi that are highly adapted to humans and do not cause diseases in non-human host. It is manifested by fever, abdominal pain, transient diarrhoea or constipation. In contrast, nontyphoidal *salmonella* strains infect a wide range of animal hosts, including poultry, cattle, and pigs, and usually cause per-acute septicaemia, acute enteritis or chronic enteritis. In the subclinical form of the nonthyphoidal salmonellosis, the animals may either have a latent infection or become temporary or persistent carriers [5].

In humans, salmonellosis can be acquired through direct contact with carrier domestic or wild animals or through the consumption of contaminated foods or water. Contaminated foods of animal origin are regarded as the

***Address for Correspondence:** Yalew Abiyu Senbeto, Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center, P.O. Box 25, Pawe, Ethiopia, Tel: + 0911168237; E-mail: yalew6vet@gmail.com; yalew.abiyu@eiar.gov.et*

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primary sources infection. The most common contaminated foods resulting in human salmonellosis include poultry, beef, chicken, turkey, pork, eggs, milk, and products made from them. The general symptoms of human salmonellosis are fever, diarrhea, abdominal cramps, nausea, vomiting, chills, and prostration. The diarrhoea varies from a few thin vegetable-souplike stools to massive evacuations with accompanying dehydration and usually the disease lasts for a few days and is self- limited but, occasionally the infection can be more severe and life threatening, with loss of fluid and electrolytes, especially to the sick, young infants, the elderly, pregnant women, and debilitated or immunocompromised persons [6].

In animals, salmonellosis can be acquired through ingestion of contaminated feed and water as well as by contact with carrier animals including humans. It is most common in pregnant, lactating or young mammals and birds and in areas where intensive animal husbandry is practiced. It is manifested in the animals in three major forms: enteritis, septicemia, and abortion. Enteritis caused by *salmonella* results in the passage of foul-smelling, watery feces, which may contain fibrin, mucus, and sometimes blood. When the enteric disease is severe, death may result from dehydration, electrolyte loss, and acid-base imbalance septicemic form often leads to abortion.

Salmonellosis in animals can also be asymptomatic, clinical and sub clinical. Clinical disease is most common in very young, pregnant or lactating animals, and usually occurs after stressful events such as poor sanitation, drought, malnutrition, overcrowding, unfavorable weather, stress of hospitalization and surgery, parturition, parasitism, transportation, weaning, exposure to cold, sudden change of feed, or overfeeding following a fast and concurrent viral or parasitic infections. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material.

Salmonellosis causes significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact. Mortality due to *salmonella* infections is mainly a health problem in developing countries, but morbidity due to acute *salmonella* infections also has important socio-economic impact in industrialized nations. *Salmonella* infections are very common and an important public health problem in many parts of the world.

Studies in different countries indicated that *salmonella*e are wide spread in small ruminants. Research to date, as well as unpublished reports from different health institutions in Ethiopia have indicated that salmonellosis is a common problem in humans, animals, animal food products and other foods. Although, little study has so far been done to isolate *salmonella* from goat`s feaces in Ethiopia from central parts of the country at abattoirs, there was no report regarding the status of *salmonella* from goats feaces in Eastern Hararghe.

Thus, the objective of this study was;

- To isolate non-typhoidal *salmonella* from goat feaces in Haramaya district.
- To estimate the prevalence of *salmonella* from goat feaces in Haramaya district.

Materials and Methods

Study area description

The study was conducted at Haramaya district specifically Becheke and Ifa Bate Peasant Associations (PA's). Haramaya is located in Eastern Hararghe Zone of Oromia Regional State, approximately 500 kms East of Addis Ababa and 14 km west of Harar town. The elevation of the area is about 2000 m a.s.l. and geographically it is located at 041°59'58'' latitude and 09°24'10'' longitudes with two ecological zones of which 66.5% is midland and 33.5% is lowland [7]. The mean annual temperature ranges from 10 °C to 18 °C with a relative humidity of 65%. The district receives an average annual rain fall of approximately 900 mm, with a bimodal distribution pattern, peaking in mid-April and mid-August. There are four seasons; short rainy season (from mid-March to mid-May), short dry season (from end May to June), long wet season (July to mid-October) and long dry season (end of October to February). Main pasture production is expected after the short rainy season, continuing until the end of the long wet season. Mixed type agriculture is the main occupation of the population of the area (Figure 1).

The study animals

The study animals of this study were goats of all age groups and both sexes reared in Becheke and Ifa Bate PA`s of Haramaya district.

Sample size determination

The sample size required for this study was determined using prevalence of 9.01% from previous study (Tadesse and Tessema, 2014) according to the formulae given by Thrusfield (2005). Thus, at 95% confidence interval and 5% absolute precision, the sample size was determined to be 126.

$$n = 1.962\, Pexp\,(1 - P\exp) / d2$$

$$n = \frac{1.962 \times 0.0901(1 - 0.0901)}{(0.05)} = 126$$

Study design and sampling method

A cross-sectional study involving microbiological analysis was conducted from November 2014 to March 2015 with consecutive sampling to isolate Non Typhoidal *Salmonella* (NTS) from Haramaya district goat faeces. Becheke and Ifa Bate PA`s were purposively selected from the study area district based on goat population potential and accessibility to road. List of small holder farmers rearing goats was obtained from each PA`s and few small holder farmers were selected using systematic random sampling method. Simple random sampling was used to select goats from which fecal sample was collected.

Study methodology

Sample collection and transportation: The feacal samples were

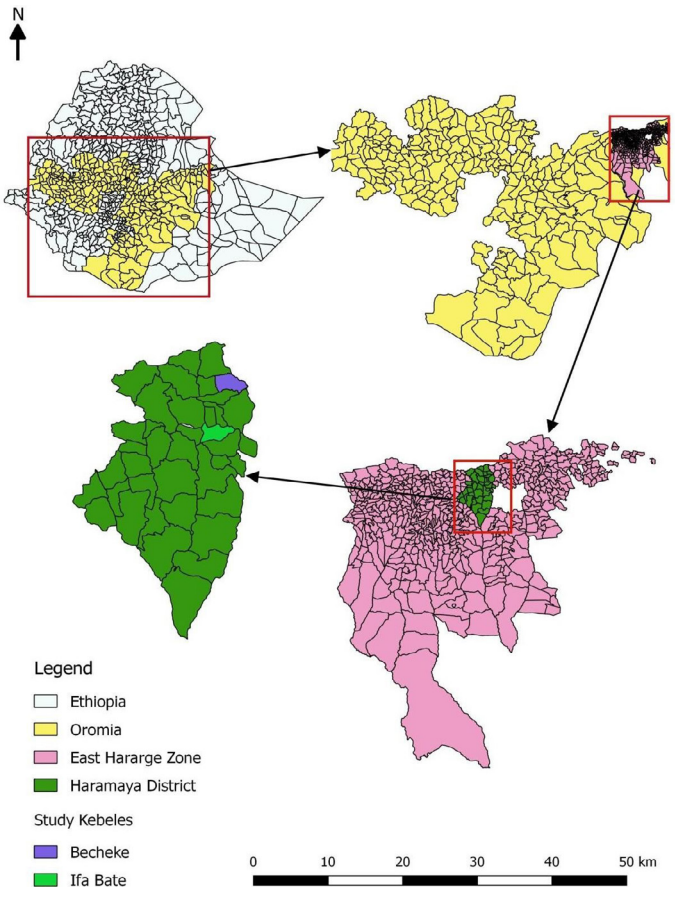


Figure 1. Study area map.

directly collected as aseptically as possible from the rectum into a sterile bottle using sterile hand gloves and labeled. The labeled samples were put into an ice box and transported to Haramaya University, College of Veterinary Medicine laboratory for microbiological analysis. The samples were processed separately within a maximum of four hours of collection up on arrival. During sampling, factors such as species, age, sex, source, housing conditions of the goats, and date of the sample collection and address of the owners were recorded. Age of the goats was determined by asking the owner and dentition. The study goats were categorized based on age as young (≤ 6 months), adult (> 6 months to 24 months) and old (> 24 months) [8].

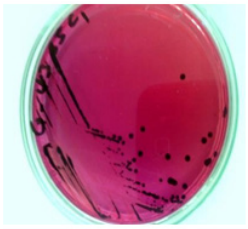
Isolation of *salmonella*: Isolation of the *salmonella* was done based on International Organization for Standardization (ISO 6579) recommendations for detection of *salmonella* species in animal feces and environmental samples [9] with some modifications. Bacteriological Medias used for the study were prepared according to manufacturer's instructions. In brief, 25 grams of individual faecal samples were thoroughly crushed using wooden spatula in the sampling bottle.

The crushed samples were transferred to separate sterile plastic containers containing 225 ml buffer peptone water (BPW) (Oxoid, CM 0509, Basingstoke, Hampshire, England) and incubated at 37 °C for 24 hrs. Samples smaller than 25 grams, were pre-enriched in a ratio of 1 gram of the sample to 9 ml of BPW. The culture media were well mixed using vortex and 1ml of the culture was transferred to sterile tube containing 10ml Selinite Cysteine (SC) (Difco TM, Becton, Dickinson, USA) broth and incubated at 370C for the next 18-24 hrs. Another 0.1 ml of the culture broth was also transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium with soya (RVS broth) (LABM, LAB 86, Lancashire, UK) and incubated at 41.5 °C for 24 hrs. Then, a loop full of inoculum from both the SC broth and RVS broth were inoculated onto Salmonella-*Shigella* Agar (SSA) (LABM, LAB 052, Lancashire, UK), Xylose-Lysine Deosoxycholate (XLD) (Oxoid, CM 0469, Basingstoke, England) agar and Brilliant Green Agar (BGA) (Difco TM, Becton, Dickinson, USA) plates and incubated at 370C overnight to allow the development of discrete *salmonella* colonies [10]. The incubation was prolonged to 48hrs for those that did not show any growth during the 24hrs incubation.

Biochemical confirmation of *salmonella*: Two to three typical *Salmonella* colonies, having a slightly transparent zone of reddish color and a black center from XLD, colorless colonies with black center from SSA and pink colonies with in red medium from BGA [9] were sub-cultured on nutrient agar (Oxoid CM 0003, Basingstoke, England) and incubated at 37 °C for 24hrs. Afterwards, the culture was confirmed biochemically using KLigler Iron Agar (KIA) (LABM, LAB 059, Lancashire, UK), O- Nitrophenyl-beta-D-Galactopyranoside (ONPG), Lysine decarboxylase (LDC), Urease, Indole and citrate tests. ONPG, Urease, LDC and Indole tests were done by using diagnostic tablets (DIATABS) (DiatabsTM, Rosco diagnostica) and citrate test was done using Simmon`s citrate agar (Difco, Detroit, USA). Three test tubes were labeled with the sample code, the DIATABS and date of incubation. Physiological saline (0.9%) was prepared by dissolving 0.9g sodium chloride in 100ml distilled water and 1 ml of the 0.9% physiological saline was added to the labeled tubes (Figure 2).

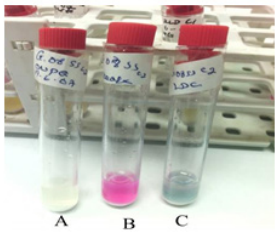
Salmonella suspected colonies were taken from Nutrient agar using sterile cotton swab and a dense suspension was made by inoculating tubes holding the saline solutions. The suspension was compared with 4Mc Farland Standard and adjusted to the Mc Farland solution. Then, ONPG, Urease and LDC DIATABS were consecutively added to the suspension and the tubes with Urease and LDC were covered with 3 drops of mineral oil to create anaerobic condition. The tubes were closed with their lids and incubated at 360c for 4-24 hours. After 24 hours incubation time, ONPG, Urease and LDC results were recorded and 3drops of Kovac`s reagent was added to LDC tube for Indole test. Finally, the reagent was thoroughly mixed with the culture by shaking and the indole result was recorded after 3 minutes of mixing. Colonies producing an alkaline slant and acid butt with hydrogen sulphide production on KIA, positive for LDC (blue color), negative for urease (orange or yellow color), negative for indole test (yellow- brown ring) and positive for citrate utilization test (blue color) were considered to be *Salmonella*- positive [9].

Citrate medium



Salmonella colonies (Black colonies) on XLD agar

KIA positive



ONPG Positive (A), urease positive (B) and LDC positive (C)

Figure 2. Pictures are showing *salmonella* colonies and biochemical tests.

Ethical considerations: In this study all ethical procedures for sampling, enrollment and sample collection were followed. Prior to animal sample collection, the objective of the study was discussed and informed consent was obtained from the owners.

Data analysis

The data was entered into a Microsoft Excel spread sheet and transferred to STATA version 11.0 for analysis (Stata Corp, 2009. Stata Corp. 4905 Lakeway Drive College station, Tx77845, USA) Fisheries exact test was used to compute the association between the study variables (sex, age, Peasant associations and housing). Statistically significant difference between the variables was considered if the calculated p-value is less than 0.05 at 95% confidence interval.

Results

Out of the total 126 goats fecal samples examined, 4 (3.2%) were positive for *salmonella*, 3 (4.9%) being from Ifa Bate and 1(1.5%) from Becheke PA`s. However, there was no significant statistical association (p=0.286) between the two PAs. Age wise prevalence of *salmonella* was found to be higher in young goats 1(8.3%), followed by old 2(5.3%) and adult 1(1.3%) goats with no significant statistical association between the age groups. The prevalence of *salmonella* was also found to be slightly higher in female goats and goats housed separately from humans than those housed with humans (p-value>0.05) (Table 1).

Discussion

In present study the prevalence of *salmonella* in goat's feaces was found to be 3.2% which is in an agreement with the prevalence range of 1-18.8% *salmonella* in goats. The result of this study is also in agreement with that of Munoz M, et al. [11]. However, studies conducted by Ferede B [12] in Diredawa Municipal Abattoir, and [3] in Mojo Export Abattoir documented a prevalence of 17.7% and 11.7% which are higher than the current study. This difference may be attributed to the difference in sample type, sampling procedures, cross contamination and bacteriological techniques employed in detecting the organism. The differences might also be due to the fact that the slaughtered animals could be stressed due to overcrowding, starvation, transportation, and slaughtered without stay in lairage. When animals are starved, *Salmonella* can survive and multiply in the rumen. Furthermore, healthy carriers intermittently excrete only a few *Salmonella*, unless they undergo some kind of stress such as transportation. Therefore, the low prevalence of *Salmonella* could be associated with low excretion of *Salmonella* due to absence of exposure

Table 1. Prevalence of *salmonella* in goat feaces by different risk factors.

Risk Factors		No. Observed	No. Positive (%)	CI (95%)	$_x$2	P value
PA	Ifa Bate	61	3(4.9)	1-13.7	0.354	0.286
	Becheke	65	1(1.5)	0.04-8.3		
	Total	126	4(3.2)	0.87-7.9	0.354	0.286
Age	Young	12	1(8.3)	0.21-38.5	0.191	-
	Adult	76	1(1.3)	0.03-7.1		
	Old	38	2(5.3)	0.6-17.7		
	Total	126	4(3.2)	0.87-7.9	0.354	0.286
Sex	Female	86	3(3.5)	0.7-9.9	1	0.621
	Male	40	1(2.5)	0.06-13.2		
	Total	126	4(3.2)	0.87-7.9	0.354	0.286
Housing	Together with human	81	1(1.2)	0.03-6.7	0.13	0.13
	Separated from human	45	3(6.7)	1.4-18.3		
	Total	126	4(3.2)	0.87-7.9	0.354	0.286

to predisposing factors such as overcrowding, starvation and transportation [4,12].

Even though, the finding of the present study was in similar and nearly similar with studies conducted by Zubair A and Khalid SI [4] who reported 3.9%, 2% and 3.8% *Salmonella* prevalence, it was larger than the reports of Dabassa and Bacha and Mahmood, who documented 1.7% and 0.3% prevalence in Jima town and Pakistan respectively. This might be associated with the difference in agro ecology, sample types and tests of the bacteriological detection as well as the differences of occurrence and distribution of *Salmonella*.

Large numbers of *salmonella* were isolated from Ifa Bate 3(4.9%) than Becheke 1(1.5%), with no significant differences (p-value>0.05) between two PAs. This could be due to difference in occurrence and distribution of *Salmonella* in the study population and due to small sample size and/or difference in sample size of goats from the PA`s regardless of test samples and methods of detection. This study also recorded more *Salmonella* isolates from female goats than male with no significant differences. This could be due to difference in sample size of male and female study goats. Even though there was no significant association (P- value >0.05) the age groups, there was also higher prevalence of *Salmonella* in young goats in the current study.

Age wise prevalence of *salmonella* was found to be non-significantly higher in young goats 1(8.3%) with no significant statistical association between the age groups. The difference in the prevalence among the age groups might be due to variation in the response to infection with Salmonella species among the age groups and the immunological status of the animal which is dependent on colostrum intake in young animals, previous exposure to infection and exposure to stressors. Precipitating factors like transport, concurrent disease, deprivation of food and parturition may also lead to higher occurrence of the disease in young and old animals than adult ones.

The prevalence of *salmonella* was also found to be non-significantly higher in female goats (3.5%) than male goat (2.5%). The higher prevalence in female goats may be attributed to their higher exposure a result of stress from pregnancy and lambing/ kidding, it might also be resulted from to the variation in the number of male and female samples tested. Goats living alone in separated house form human showed higher prevalence of *Salmonella* isolates (6.7%) than those living with human in the same house (1.2%) (p- Value >0.05). This condition might be due to the fact that there is higher hygienic practice of those farmers housing the goats with themselves and ignorance of frequent cleaning of the separate house of the goats. Frequent cleaning of the house reduces risk of contamination and infection with the bacteria.

Conclusion

Animals are the main reservoirs for non-typhoidal *Salmonella* and they shed the pathogen along with their feaces. The results obtained in the present study (3.2%) imply that goat faeces could be potential reservoirs of *Salmonella* and could possibly cause infection as a result of contamination of food products. The causative agent being isolated from fecal sample is also important in demonstrating that apparently healthy goat may act as reservoirs. The current study indicates non difference in prevalence of *Salmonella* indicating endemic occurrence of the disease in the area. Although the overall prevalence of *Salmonella* reported by the present research work is not considered high, it could not be neglected due to its zoonotic importance. In general, control and prevention of salmonellosis in live animals require implementation of risk reduction strategies throughout the food chain.

Recommendations

Thus, based on the above conclusion the following recommendations are forwarded:

- Further study on isolation, identification and antimicrobial susceptibility of non-typhoid *salmonella* should have to be done.
- Prevention and control measures should be practiced without negligence.

Acknowledgement

None.

Conflict of Interest

None.

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