International Journal of Microbiological Research 8 (1): 09-18, 2017
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2017.09.18

# Isolation of Nontyphoidal *Salmonella* in Cattle, Sheep and Goats among Three Different Agro-Ecologies of Eastern Hararghe, Ethiopia

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**Abstract:** Nontyphoidal Salmonella (NTS) are zoonotic pathogens and a wide variety of animals have been identified as reservoirs. This study was conducted to estimate the prevalence of NTS and to identify the risk factors associated with NTS in cattle, sheep and goats among three different agro-ecologies of Eastern Haraghe, Ethiopia. A total of 690 faecal samples were collected including 202 from cattle, 236 from sheep and 252 from goats. The samples were examined for the presence of NTS following standard techniques and procedures outlined by the International standardization for organization (ISO 6579). From the total animal examined 5.07% were positive for NTS, of which 6.93% were cattle, 4.70% were sheep and 3.97% were goats. There was significant difference ($p<0.05$) in the overall prevalence among age groups. The prevalence of NTS in highland, midland and lowland agro-ecologies were 1.43, 3.85 and 18.89%, respectively and there was a statistical significant difference ($p<0.05$) among agro-ecologies. Results of the present study indicated that age and agro-ecology were a crucial factors related with the prevalence of Salmonellosis. Though, the overall prevalence reported by the current study is not considered to be high, but it could not be neglected because of its zoonotic importance. So, control and prevention of salmonellosis in live animals and implementation of risk reduction strategies should be implemented.

**Key words:** Babille · Cattle · Eastern Ethiopia · Goat · Haramaya · Jarso · NTS · Sheep

## INTRODUCTION

Salmonellosis is an infection caused by *Salmonella* bacteria that mainly affects cattle, sheep, goats, chickens and humans. The species associated with salmonellosis in humans can be divided into those causing typhoid fever, which are transmitted from human to human and nontyphoidal species, for which transmission through contaminated food is thought to cause 85% of human cases [1].

Nontyphoidal *Salmonella* (NTS) are zoonotic pathogens and a wide variety of animals have been identified as reservoirs [2]. Infection in animal is of importance because of the direct economic consequences of salmonellosis attributable to mortality and morbidity [3]. Food animals harbor a wide range of *Salmonella* serotypes and so act as source of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis [4, 5].

The global burden of non typhoidal *salmonella* gastroenteritis has been estimated to be 93.8 million cases of gastroenteritis each year, with 155 000 deaths [6]. The threat of epidemic infections has increased due to the globalization of the food supply and the increased movements of people, animals and goods within and between countries [2, 7]. Apart from the morbidity and mortality costs in humans and animals, restrictions to trade and discard of contaminated food are important socioeconomic problems of the bacteria [8].

Infections with salmonellosis occur when a susceptible animal ingests the bacteria. Cattle ingest feed or water that has been contaminated with faeces from animals shedding the organism. So, infected cattle shed the bacteria and act as source of infection [9]. Small ruminants, such as sheep and goats, are also potential carriers of Salmonella [10].

In Africa, NTS has consistently been reported as a leading cause of bacteremia among immunocompromised people, infants and newborns [11]. However, it is usually

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difficult to evaluate the situation of salmonellosis in developing countries; it is due to the very limited scope of studies and lack of coordinated epidemiological surveillance system [12]. In addition to this, under reporting of cases and the presence of other disease considered to be of higher priority may have over shadowed the problem of salmonellosis in some developing countries including Ethiopia [13]. The increased global population coupled with mass production of animal and human food could worsen the problem [14].

Ethiopia has the largest animal population in Africa and the living standard of the population is generally favorable for the transmission of pathogens from animals to humans and the vice versa. Despite salmonellosis being one of the important zoonotic diseases, surveillance and monitoring systems are not in place and the temporal and spatial distributions of the serotypes are not described [15].

Infected animals are the source of the organisms [16, 17]. The faecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain [18]. Members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and in the intestinal tracts of animals [19]. People can become infected following a failure of personal hygiene after contact with infected animals and or other infected people [20]. *Salmonella* also has substantial financial and social impacts; it is estimated to cost nations billions of dollars annually [21].

In general, the primary sources of salmonellosis are considered to be food producing animals such as cattle, sheep and goat [22]. The pathogens are mainly disseminated by trade in animals and uncooked animal food products [23]. Hence the disease is common in intestinal illness which is caused by numerous *Salmonella* serovars with clinical manifestations that vary from severe enteric fever to mild food poisoning [24] both in humans [25] and animals [21].

NTS represents an important human and animal pathogen worldwide [26]. A number of studies conducted on poultry, pig, poultry meat, minced beef and humans in Ethiopia showed that *Salmonella* are prevalent in various slaughtered animals and their meat products [27-33] and human beings [34]. There is paucity of well documented information on the prevalence and occurrence of salmonellosis in live animals such as; cattle, sheep and goats in Ethiopia especially in eastern part of the country. Therefore the objectives of this study was designed to estimate the prevalence of NTS and to identify the risk factors associated with NTS from faeces of cattle, sheep and goats among three different agro-ecologies of Eastern Hararghe, Ethiopia.

## MATERIALS AND METHODS

**Study Area:** This study was conducted in three districts having different agro-ecologies of Eastern Hararghe zone of Oromia Regional State. According to the report of CSA (central statistical agency) [35], East Hararghe zone is one of the 18 zones of Oromia Regional State, which is the largest among nine regional states of Ethiopia. Including the study area, Haramaya, Babille and Jarso districts, the zone has fifteen administrative districts.

Table 1: Total livestock population by district and species

| | | Districts | | | |
|---|---|---|---|---|---|
| No | Species | Haramaya | Babille | Jarso | Total |
| 1 | Bovine | 107090 | 48500 | 63244 | 218834 |
| 2 | Ovine | 79950 | 11835 | 41521 | 133306 |
| 3 | Caprine | 125145 | 21501 | 67130 | 213776 |
| | Total | | 565916 | | |

Sources: Haramaya, Babile and Jarso districts animal health, 2014 livestock population data [36-38].

**Study Design:** A cross sectional study was undertaken among three different agro-ecologies from July 2015 to January 2016. These were highland (Jarso district), midland (Haramaya district) and lowland (Babille district) of Eastern Hararghe.

**Study Population:** The sampling units of the study were cattle, sheep and goats of all age groups and both sexes reared in three different agro-ecologies.

**Sampling Method and Sample Size:** Three districts were selected purposively based on their agro-ecology and accessibility of logistics. From which a total of six peasant associations (PAs), two PAs from each district which are representatives of the three different agro-ecologies were randomly selected and the households was selected using a systematic sampling method. The intervals between households were made after calculating the $K^{th}$ value then studied animals were selected using simple random sampling method. Most of the study animals shared the same households in the study areas. The sample size required for the study was calculated by the formula given by Thrusfield [39].

$$n = \frac{1.96^2 \, P_{exp} \, (1- P_{exp})}{d^2}$$

where:
n= required sample size
$P_{exp}$ = expected prevalence
d = desired absolute precision

Since there was no previous prevalence estimate in this study area, for determination of sample size, a compiled and aggregated prevalence of *Salmonella* in food animals in Ethiopia was used. The meta-analysis report indicated the national prevalence of *Salmonella* in cattle, sheep and goats was 7.07, 8.41and 9.01% respectively [15]. Therefore, 95% confidence interval, 5% precision and previously mentioned expected *Salmonella* prevalence of cattle, sheep and goats were used to estimate the sample size. Accordingly, the number of cattle, sheep and goats needed to determine the prevalence of *Salmonella* was determined to be 101, 118 and 126 respectively. However, to increase precision the sample size was doubled to 202, 236 and 252 for cattle, sheep and goats respectively. Since the study included three different districts with different agro-ecologies, the overall sample size was proportionally allocated to each district.

In the actual sample collection, two Peasant associations (PAs) were randomly selected from each districts. The number of animal faeces sampled from different PA's was based on the total population of animals in the selected PA's within each district.

**Sample Collection:** Fresh faecal samples were directly taken from the rectum of cattle, sheep and goats with sterile gloves. These samples were collected into sterile bottles free from disinfectant residues. During sampling, age of each species of animal and address of the owners were recorded. Ages of ruminants were determined based on the eruption of teeth. Finally, the bottles were labeled and put into an ice box with ice packs and transported to Haramaya University, College of Veterinary Medicine Microbiology Laboratory for microbiological analysis. Then the examination was performed immediately.

**Isolation and Identification:** Isolation and identification of *Salmonella* were done based on International Organization for Standardization (ISO) recommendations for detection of *Salmonella* species in animal faeces and environmental samples [64]. The bacteriological media were prepared according to manufacturer's recommendations.

**Pre-enrichment and Selective Enrichment:** Twenty five grams of fresh faecal samples collected from individual animal were crashed in sampling bottles using individual sterile wooden spatula. The crashed samples were added into a disposable plastic container containing 225ml of buffered peptone water (Oxoid, CM 0509, Basingstoke, Hampshire, England) as a pre-enrichment medium to obtain 1 part sample and 9 part buffer. The samples were mixed well by shaking and incubated at 37°C for 16 hours. Rappaport Vassiliadis soy peptone (RVS) broth and selenite cysteine broth were used for selective enrichment of samples. About 0.1 ml of the pre-enriched sample was transferred using a pipette into a tube containing 10 ml of Rappaport Vassiliadis soy peptone broth (RVS; LAB$^M$, LAB 86, Lancashire, UK) and incubated at 41.5°C for 18 hours. Another 1 ml of the pre-enriched broth was transferred in to a tube containing 10 ml selenite cysteine broth (SC: Difco $^{TM}$, Becton, Dickinson, USA) and incubated at 37°C for 18 hours.

**Plating out and Identification:** Xylose lysine deoxycholate (XLD) agar and *Salmonella Shigella* Agar (SS) plates were used for plating out and identification. A loop full of inoculums from each RV and SC broth cultures were plated onto XLD (XLD; Oxoid, CM 0469, Basingstoke, England) and *Salmonella Shigella* Agar (SSA; LAB$^M$, LAB 052, Lancashire, UK) plates prepared according to the manufacturer direction. The plates were incubated at 37°C for 18-24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies. If growth was slight or if no typical colonies of *Salmonella* were present on agar plates, they were re-incubated at 37°C for another 18-48hr. Typical colonies of *Salmonella* grown on XLD-agar has a slightly transparent zone of reddish color and a black center, a pink-red zone was also seen in the media surrounding the colonies. On SS Agar *Salmonella* shows a black center with lightly pale zone. For confirmation, about five presumptive or suspected colonies were selected from the selective plating media. If the suspected colonies on each plate are fewer than five, all the colonies were selected. Then the selected colonies were streaked onto the surface of nutrient agar plates (Oxoid CM 0003, Basingstoke, England) and incubated at 37°C for 24hrs.

**Biochemical Identification:** All suspected *Salmonella* colonies were picked from the nutrient agar and confirmed biochemically by using Kligler Iron Agar (KIA) (KIA; LAB$^M$, LAB 059, Lancashire, UK), citrate (Difco, Detroit, USA), O-Nitrophenyl-beta D-galactopyranoside (ONPG), Lysine Decarboxylase (LDC), urease and indole test.

ONPG, Urease and LDC tests were performed by using diagnostic tablets (DIATABS). Suspected colonies were inoculated in 1 ml of sterile saline to give a dense suspension (McFarland 4). Following this a single tab in each tubes and three drops of mineral oil was added on LDC and urease. Each tube was observed for color change following incubation at 3-24 hrs. Indole test was performed on LDC test tubes following suggestive result after adding three drops of Kovac's reagent [40, 41]. Then, the results were checked after three minutes of recapping and shaking the tubes.

*Salmonella* colonies produce an alkaline slant (red) with acid (yellow color) butt on KIA with or without hydrogen sulphide production, positive for lysine and negative for urease [42].

**Data Management and Analysis:** Microsoft Excel was used for data entry and management. Descriptive statistics such as percentage, proportion, frequency distribution were applied to compute the data. Univariable logistic regression was used to fit the multivariable logistic regression. To determine the strength of association, odds ratios were calculated. The data was analyzed by comparing proportions, using Pearson's chi-square or Fisher's exact test based on the number of observations per contingency table cells. Statistically significant associations between variable were considered when the p value is less than 0.05. All statistical analysis was performed using SPSS version 20 software package.

**Data Quality Control:** All laboratory procedures including media preparation, procedures of each testing technique was done according to manufacturer production guideline. Sterilization procedures and collection and handling of specimens were carried out in accordance with standard protocols [43]. The necessary reagents and samples were checked for contamination each time before handling and kept in proper condition [44].

## RESULTS

**Overall Prevalence of Nontyphoidal *Salmonella*:** Out of the total 690 animals randomly selected and examined for the prevalence of nontyphoidal *Salmonella* among the three different agro-ecologies; highland (Jarso district), midland (Haramaya district) and lowland (Babille district) of eastern Hararghe, Ethiopia, 35 (5.07%) were found to shed the organism in their faeces. In addition wider range of prevalence of NTS has been observed among three agro-ecologies of interest. Accordingly, the prevalence ranges from 1.43 to 18.89% in the study area. The detail information on the prevalence of NTS among species in different agro-ecologies is given in Table 1.

Table 1: Prevalence of *Salmonella* among examined animals in different agro-ecologies

| Agro-ecologies | Animal species | Examined | No. positive | Prevalence (%) | 95% CI |
|---|---|---|---|---|---|
| | Cattle | 44 | 7 | 15.91 | 6.6-30.0 |
| Lowland | Sheep | 21 | 5 | 23.81 | 8.2-47.2 |
| | Goat | 25 | 5 | 20.00 | 6.8-40.7 |
| Total | | 90 | 17 | 18.89 | 11.4–28.5 |
| | Cattle | 99 | 6 | 6.06 | 2.3-12.7 |
| Midland | Sheep | 142 | 5 | 3.52 | 1.2-8.0 |
| | Goat | 149 | 4 | 2.68 | 0.8-6.9 |
| Total | | 390 | 15 | 3.85 | 2.1–6.3 |
| | Cattle | 59 | 1 | 1.69 | 0.04-9.1 |
| Highland | Sheep | 73 | 1 | 1.37 | 0.03-7.4 |
| | goat | 78 | 1 | 1.28 | 0.03-6.9 |
| Total | | 210 | 3 | 1.43 | 0.3–4.1 |

Table 2: Prevalence of *Salmonella* among animal species

| Species | No. examined | No. positive | Prevalence (%) | 95% CI |
|---|---|---|---|---|
| Cattle | 202 | 14 | 6.93 | 3.8-11.3 |
| Sheep | 236 | 11 | 4.66 | 2.3-8.2 |
| Goats | 252 | 10 | 3.97 | 1.9-7.2 |
| Total | 690 | 35 | 5.07 | 3.5-6.9 |

Similarly, among animal species the highest prevalence was found in cattle when compared to sheep and goats in midland and highland districts under the current investigation. Table 2 shows the prevalence of NTS among species.

To have an insight on the overall prevalence of *Salmonella* among both sex group of livestock (three species of interest) similar analysis have been made. Consequently 5.8 and 4.2% prevalence of *Salmonella* was proved to be in female and male animals under investigation, respectively. The overall prevalence of *Salmonella* among different age groups was also calculated and is shown in Table 3.

**Risk Factors Associated With Nontyphoidal *Salmonella*:** While calculating the overall prevalence, analysis of certain risk factors was made to identify the difference in the overall prevalence of nontyphoidal *Salmonella* among different age groups, sex, species and agro-ecologies.

Accordingly, the result taken from the analysis of overall prevalence difference between agro-ecologies showed higher prevalence of *Salmonella* in lowland (Babille district), while the lowest in the highland (Jarso district). Statistically significant difference ($p<0.05$) was observed among agro-ecologies. The prevalence

Table 3: Prevalence of *Salmonella* among different age groups and sex in cattle, sheep and goats

| | Species | No. examined | No. positive | Prevalence (%) | 95% CI |
|---|---|---|---|---|---|
| Age | Young | 191 | 13 | 6.81 | 3.7-11.4 |
| | Adult | 263 | 4 | 1.52 | 0.4-3.8 |
| | Old | 236 | 18 | 7.63 | 4.6-11.7 |
| | Total | 690 | 35 | 5.07 | 3.5-6.9 |
| | Cattle | 82 | 6 | 7.31 | 2.7-15.2 |
| Male | Sheep | 113 | 5 | 4.42 | 1.4-10.0 |
| | Goats | 114 | 2 | 1.75 | 0.2-6.2 |
| | Total | 309 | 13 | 4.2 | 2.2-7.1 |
| | Cattle | 120 | 8 | 6.67 | 2.9-12.7 |
| Female | Sheep | 123 | 6 | 4.88 | 1.8-10.0 |
| | Goats | 138 | 8 | 5.79 | 2.5-11.1 |
| | Total | 381 | 22 | 5.8 | 3.6-8.6 |

Table 4: Difference in the prevalence of *Salmonella* based on different risk factors

| Risk factors | Category | Prevalence (%) | 95 % CI | p value |
|---|---|---|---|---|
| | Midland | 3.85 | 2.1–6.3 | |
| Agro-ecologies | Lowland | 18.89 | 11.4–28.5 | 0.00 |
| | Highland | 1.43 | 0.3–4.1 | |
| Species | Cattle | 6.93 | 3.8-11.3 | |
| | Sheep | 4.66 | 2.3-8.2 | 0.338 |
| | Goats | 3.97 | 1.9-7.2 | |
| Age | Young | 6.81 | 3.7-11.4 | |
| | Adult | 1.52 | 0.4-3.8 | 0.004 |
| | Old | 7.63 | 4.6-11.7 | |

of *Salmonella* among PA's of the same districts (agro-ecologies) was also analyzed to see whether significance association is presented or not. However, there was no statistical significant difference ($p>0.05$) on the prevalence of *Salmonella* among PA's. Even if, the differences in the prevalence of *Salmonella* among species were detected in the current study, which was lowest in goats and highest in cattle, however there was no statistically significant difference ($p>0.05$) observed in the prevalence between the three species. In addition to sex, species and agro ecology, age was found an important risk factor significantly associated with the prevalence of *Salmonella* in the study areas (Table 4).

There was no statistically significant difference ($p>0.05$) in Prevalence of *Salmonella* in cattle, sheep and goats, among the three agro-ecologies. In addition, analysis of the data also showed no statistically significant difference ($p>0.05$) in the prevalence of *Salmonella* among the sex groups of cattle, sheep and goats (Table 5).

Attempt univariable and multivariable logistic regression were made to evaluate the association and strength of association between the variables and *Salmonella* shading. Accordingly, only age and districts were found to be significantly associated following multivariate logistic regression (Table 6).

Table 5: Prevalence of *Salmonella* based on sex and species in each agro-ecology as risk factors

| Risk factors | | Category | Prevalence(%) | 95 % Confidence Interval | P value |
|---|---|---|---|---|---|
| Species | Cattle | Female | 6.67 | 2.9–12.7 | 0.858 |
| | | Male | 7.31 | 2.7–15.2 | |
| | Sheep | Female | 4.88 | 1.8–10.0 | 0.558 |
| | | Male | 4.42 | 1.4–10.0 | |
| | Goats | Female | 5.79 | 2.5–11.1 | 0.118 |
| | | Male | 1.75 | 0.2-6.2 | |
| Agro-ecologies: | Midland | Cattle | 6.06 | 2.3-12.7 | 0.39 |
| | | Sheep | 3.52 | 1.2-8.0 | |
| | | Goat | 2.68 | 0.8-6.9 | |
| | Lowland | Cattle | 15.91 | 6.6-30.0 | 0.74 |
| | | Sheep | 23.81 | 8.2-47.2 | |
| | | Goat | 20.00 | 6.8-40.7 | |
| | Highland | Cattle | 1.69 | 0.4-90.8 | 0.98 |
| | | Sheep | 1.37 | 0.4-73.9 | |
| | | Goat | 1.69 | 0.3-69.4 | |

Table 6: Prevalence of *Salmonella* among age groups and districts

| Variables | Variable categories | No examined | No Positive | Prevalence (%) | Adjusted OR (95% CI) | p value |
|---|---|---|---|---|---|---|
| Age | Young | 191 | 13 | 6.81 | Reference | |
| | Adult* | 263 | 4 | 1.52 | 0.2 (0.07-0.73) | 0.013 |
| | Old | 236 | 18 | 7.63 | 1.4 (0.6-3.1) | 0.388 |
| Agro-ecologies | Midland | 390 | 15 | 3.85 | Reference | |
| | Lowland | 90 | 17 | 18.89 | 6.1 (2.8-13.0) | 0.000 |
| | Highland | 210 | 3 | 1.43 | 2.8 (0.85-9.95) | 0.105 |

*being an adult is curative.

## DISCUSSION

In the present study the prevalence of *Salmonella* in cattle, sheep and goats was 6.93, 4.70 and 3.97% respectively, but the difference was not statistically significant ($p>0.05$). In agreement with the current finding, there are reports that indicate a direct relationship between *Salmonella* infection rates in cattle and small ruminants, where an increase in the infection rates of the latter has coincided with high incidence of the same serotype in the former [45]. This may occur more likely in pen, grazing areas where both large and small ruminant are kept together [27].

The present study revealed 6.93% *Salmonella* prevalence in cattle from the study area. This finding is in consistent with the range cited [46], who indicated that the prevalence of *Salmonella* in cattle falls between 0.3 and 11.6%. The current finding is also relatively comparable despite different in methodology with the reports by Fegan *et al*. [47] and Fagerberg [48] who reported 6.8 and 9% in Australia and USA respectively from cattle faeces. However, the current study revealed higher prevalence of *Salmonella* than the findings of 2% in Iraq [49] and 1.8% in Addis Ababa and its surroundings [50]. Similarly 1.9 [13], 3.1 [27] and 3.3% [31] prevalence of *Salmonella* from cattle faeces was reported in Addis Ababa, Debre-Zeit and Jimma abattoirs respectively. In contrary, the finding of the current study is lower than 34.2% prevalence [51] from adult diarrheic cattle in California, 41.3% [52] in Maltese Island of Gozo and 52% [53] in Burkina Faso. Such a difference with the current study might be attributed to sampling of diarrheic animals that are suspected to have high incidence of *Salmonella* infection [51, 53], while others had been held for several days (over the weekend) on market, transportation and lairage before slaughter and were chosen as they were more likely to carry *Salmonella* and also may be based on animal husbandry practices [47].

The prevalence of *Salmonella* in sheep was 4.7%. This finding is in agreement with the 4.8% prevalence of *Salmonella* from faeces of apparently healthy slaughtered sheep [54] and [28] at Addis Ababa municipal and Modjo modern export abattoirs respectively. The current report is also supported by earlier observation of 4% prevalence of *Salmonella* in India [55]. In contrast, 14.3% prevalence was reported from faeces of sheep at Bangladesh farm [56], which is higher than the current report. However, Studies in Iraq from faeces of sheep at Zakho abattoir [49] indicated a prevalence of 2.5% and also 3.2% in Egypt [57]. In Ethiopia, 2.1% from faeces of slaughtered sheep at Debre-Zeit abattoir [58] and 3.3% prevalence of *Salmonella* in faeces of sheep [31] were also indicated. The difference in the reported prevalence's could be associated with the sampling plan and procedures, sample type, distribution of *Salmonella* in examined sheep and the method of detection employed. It is also known that keeping animals to be slaughtered in the abattoir's waiting pens crowded could facilitate the excretion and transmission of infection among the animals [58]. In addition to this, the difference in the prevalence reported could be due to differences in study sites and animal populations.

In agreement with the current finding which is 3.97% prevalence of *Salmonella* in goats, 3.3% [58] at Debre-Zeit abattoir was also reported. Relatively lower prevalence of *Salmonella* in goats was reported by different authors in and outside Ethiopia. Accordingly, 0.00% [31] prevalence of *Salmonella* from faeces of goat in Jima abattoir and 0.01% [59] prevalence of *Salmonella* was also reported in Pakistan from diarrheic adult goat faeces. While, 2% prevalence in Addis Ababa municipal abattoir [24], Modjo modern export abattoirs [28] and from Iraq [49] and 2.3% in Zambia was reported [60].

In this study, the difference in the prevalence of *Salmonella* in species among different agro-ecologies was not statistically significant. However, higher prevalence of *Salmonella* was detected in species of lowland while the lowest prevalence was detected from species of high land agro-ecology.

There was no any significance difference detected among sex in cattle, sheep and goats. In both sheep and goats relatively higher prevalence of *Salmonella* was detected from females than males. Unlike sheep and goats, higher prevalence of *Salmonella* in cattle was detected from male 7.3% than female 6.7%. This shows that as there was no any consistency in the prevalence of *Salmonella* among sexes, it might be due to variation in the number of samples examined between male and female.

In this study, the difference in the overall prevalence of *Salmonella* among age groups was statistically significant ($p<0.05$). The prevalence of *Salmonella* in young was 6.8%, in adult 1.5% and in old 7.6%. Adult animal's shade *Salmonella* 5 times lower than young and old animals and they are more protective. The difference in the prevalence of *Salmonella* among age group may be due to variation in the response to infection with a *Salmonella* species challenge dose among age groups and the immunological status of the animal which is dependent on colostrum intake in neonates (young animals), previous exposure to infection and exposure to

stressors, particularly in older animals. It is also some precipitating factors such as transport, concurrent disease, acute deprivation of food, or parturition may cause the disease in old and young animals than adult [21].

Greater susceptibility in the very young may be the result of high gastric pH, absence of a stable intestinal flora and limited immunity [61]. The prevalence of *Salmonella* infection was increased with the increase in age [32]. The current study result signifies higher prevalence of *Salmonella* in old animals. In agreement with the present study, an increase in the prevalence of *Salmonella* with increase in age [32] was reported. In contrast, a negative relationship between age and disease which was attributed to the delay in development of immunity in younger animals thereby increasing their susceptibility to infection compared to the adult [62].

A significant association ($p<0.05$) among the three different agro-ecologies; lowland, midland and highland were observed with a prevalence of 18.89, 3.85and 1.43% respectively. However there was no any significant difference between the prevalence of *Salmonella* among PA's. A highest prevalence of *Salmonella* in diarrheic animal of low land areas in the temperature range from 25-30 ºC was reported [59], which turned out to be an ideal environment temperature for the occurrence of *Salmonella* in diarrheic animals. This report is in relation with highest prevalence of *Salmonella* in lowland agro-ecology (Babille district), which is low land having annual temperature range from 24-28 ºC. Factors related to agro-climatic situations with quantity and quality of feed, along with temperature of the area and the grazing pattern of the animals are vital for the development of infestation which may influence the prevalence of *Salmonella* in the area [59]. The incidence of salmonellosis in animals may change within a geographical area over a period of years [63]. It supports the present findings that have different agro ecologies or geographical location.

## CONCLUSIONS AND RECOMMENDATION

Nontyphoidal *Salmonella* are zoonotic pathogens and a wide variety of animals have been identified as reservoirs. Food animals harbor a wide range of *Salmonella* serotypes and act as source of contamination, which is of paramount epidemiological importance. Since, faeco-oral route is the major source of transmission of the disease; healthy animals harboring the organism act as a source of infection for other animals by contamination of pasture and drinking water. There was a significant increase in human salmonellosis in recent years and foods of animal origin are an important source of human infection, in many countries including Ethiopia.

In the present study among species statistical significant difference were not detected. Among different risk factors included in this analysis, age and agro-ecology of the study area were found to be significantly associated with prevalence of *Salmonella.* Though, the overall prevalence reported by the current study is not considered high, but it could not be neglected because of its zoonotic importance. Generally, to control and prevent salmonellosis in live animals and animal products, risk reduction strategies should be implemented throughout the food chain. Therefore, based on the findings of the present study, the followings recommendations are forwarded:

- Care should be taken to those animals purchased from lowland areas for slaughter purpose.
- Avoid mixing of different age groups of animals together during grazing and housing.
- Fecal contamination of feed and water supplies should be prevented.
- Comprehensive educational programs for owners of the animals through different meeting.

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