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Isolation, Identification and Antimicrobial Sensitivity Profile of Salmonella Isolates from Diarrheic Calves in Sebeta Town Dairy farms, Central Ethiopia

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Abstract

A cross-sectional study was conducted in dairy farms of Sebeta town from November 2018- May 2019 with a purposive sampling method to isolate, identify *Salmonella* isolates from diarrheic calves. The farms were categorized based on production levels into small (<10 animals), medium (10 to 50 animals), and large (>50 animals) farms based on the number of animals. The samples were examined for the presence of *Salmonella* following the conventional techniques of ISO standard and using OMNILOG bacterial identification system, GEN III microplate for confirmation and sub species identification. Out of 116 fecal samples examined for the isolation of *Salmonella*, of which 8 (6.89%) were positive. Among potential risk factors considered, flock size (P=0.010), calves age (P=0.023), body condition (P=0.019), colostrum feeding (P=0.023), bedding (P=0.017), calves kept in their pens with a frequency of change of bedding material (P=0.016), and feeding up to weaning (P=0.054) were closely linked with the occurrence of *Salmonella* infection. In conclusion, this study shows *Salmonella* could be considered as a potential causative agent for a diarrheic in calves. Among 8 isolates of *Salmonella*, all showed 100% susceptibility for sulphamethoxazole + trimethoprim and polymixin B, 87% to ampicillin, chloramphenicol, and tetracycline; gentamicin showed 75% susceptibility for the agent. However, only one isolate showed mono drug (tetracycline) resistance. Therefore, improved management practices and appropriate antibiotic treatment of diarrheic calves should be employed to prevent the spread of *Salmonella* infection occurring in healthy animals at Sebeta and other similar farms in Ethiopia.

INTRODUCTION

Dairy production is a critical issue in Ethiopian livestock-based society where livestock and its products are important sources of food and income. The substantial demand-supply variance in milk and milk products for the major urban centers in Ethiopia is a great opportunity for the development and flourishing of peri-urban dairy farms. Peri-urban and urban dairies are intensive production systems, which keep high-grade cows and have improved management practices. This is usually associated with increased susceptibility to disease, poor survival rate, and poor reproduction. The country has a large potential for dairy development mainly due to the large livestock population, the favorable climate for improved high yield breed, and relatively disease-free environment [1].

Newly born calves represent an important source of animal production for either meat or breeding worldwide. Calves are important assets for the replacement of cows for future dairy and beef herd sustainability. Diarrhea is one of the very common disease syndromes in neonatal calves in different countries, and this can have severe impacts both economically and in terms of animal welfare [2] Calves are at greatest risk of developing diarrhea within the first month of life, and the incidence of diarrhea decreases with age [3].

Causes of neonatal calf diarrhea are commonly associated with more than one of these agents, and the causes of most outbreaks are usually multifactorial [4]. Among these organisms, Salmonella is the main cause of calf's diarrhea as white scour diarrhea. Salmonella is a gram-negative, rod-shaped, flagellated, and motile (except *Salmonella pullorum* and *Salmonella gallinarum*) oxidase negative, facultative anaerobe and is classified under the family *Enterobacteriaceae* [5].

Calves are infected by the ingestion of the organism from the environment which is contaminated with feces from infected animals. Six hours after ingestion, the organism multiplies in the intestine and can be found in the rectum. The bacteria invade the intestinal mucosa and adhere to the small intestine epithelial cells and overcome host defense mechanisms to enable infection and cause an inflammatory response, and septicemia and pneumonia

can follow. Signs of clinical disease are those of diarrhea and dehydration [6].

Infected calves can be either clinical or subclinical, shedding *Salmonella* in their feces; thus, dairy producers need to be aware that *Salmonella* can be found on their farms within apparently healthy cows, which is important in terms of food safety risks [7]. The persistence of infection is an important epidemiologic feature of *Salmonellosis* and can be related to serotypes with which the animal is infected [8].

Incomplete course of treatment and continuous indiscriminate uses of antibacterial drugs against salmonella infection of calves might have influenced to produce a new generation of virulent and resistant type of salmonella. Although routine laboratory isolation and drug sensitivity testing are expensive and impractical, the periodical check of the profile of the drug sensitivity of organisms is more significant. However, reports on salmonella associated with calf diarrhea are very limited in Sebeta. Therefore, updated data on *Salmonella* in diarrheic calves and antimicrobial susceptibility is very important for proper selection and use of antimicrobial agents in a setting. The objective of this study was to isolate and identify *Salmonella* from diarrheic calves in the study area, determine the antimicrobial resistance patterns of *Salmonella* isolates to the selected antibiotic discs and assess the potential risk factors of *Salmonella* associated with diarrheic calves in selected dairy farms.

MATERIALS AND METHODS

Study area

The study was conducted in Sebeta town from November 2018 to March 2019. Sebeta is the administrative center of Sebeta Hawas district (Figure 1) located in the Oromia Special Zone Surrounding Finfinne City of the Oromia Region, Ethiopia. It is located 24 km southeast of Addis Ababa and lies at a latitude of 8º5440' N and longitude of 38º3717' E with an elevation of 2356 meters (7730 feet) above sea level. The average temperature is 17.4ºC and the town receives an annual rainfall of 1650 mm, the monthly precipitation is being 150 mm are mostly wet and below 30 mm mostly dry. Mixing farming system were extensively practiced in the area where this study was conducted from the dairy farms of intensively managed cattle.

Study Population

The study population included in this study were cross and local breeds of cow calves of both sex up to 6 months of age that were clinically affected with diarrhea and exhibiting signs of systemic disease (e.g., poor appetite, fever, sunken eye, systemic dehydration, and reduced suckle reflex), and profuse watery diarrhea. All diarrheic calves in dairy farms of Sebeta town were incorporated for sampling during the study period. Twenty-eight small-sized, ten medium, and nine large sized dairy farms were involved in the study period and classified as small (< 10 head of a dairy cow), medium (10 to 50 head of a dairy cow), and large (>50 head of a dairy cow) [9]. Ages of diarrheic calves were categorized into two age groups: 0-3-month age and 4-6-month age, according to Lemma *et al.* [10]. Body condition scoring of examined calves was carried out according to the method described and categorized into three scores as poor, medium, and good [11].

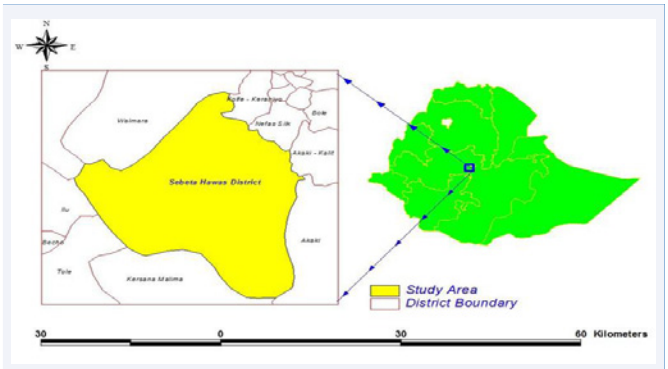

Figure 1 Map of Study Area.

Study design

A cross-sectional study was carried out to isolate, identify and detect antimicrobial susceptibility test of the Salmonella from diarrheic calves. Information about the calves was gathered by interviewing farm owners and animal health workers of selected study sites. Calves were clinically examined for the presence of diarrhea or not and fecal samples were collected for diagnostic testing [10]. At the time of sampling, the name of the farm, date of sampling, consistency of feces, age, breed, and tag number was recorded for each calf in the proper recording format.

Sampling Technique

The study areas were purposively selected and identified based on transport accessibility and geographical location and on the abundance of dairy farms to get more calves. Purposively about 25g of fecal sample was collected from non-treated diarrheic calves directly from the rectum by using sterile universal bottles after cleaning the anal area with a paper towel and beats by rectal stimulation with the index finger using disposable sterile plastic gloves. Then samples were transported in an ice box to the Bacteriology Laboratory, National Animal Health Diagnostic and Investigation Center (NAHDIC) where they were inoculated into appropriate culture media. Fecal samples were stored at 4 °C until the time of processing.

Bacterial Isolation and Identification Methods

The method used for the culture of *Salmonella* was according to the technique recommended by the International Organization for Standardization [12-13]. The bacteriological media used in different stage were prepared according to the manufacturer's recommendations. The 1 grams of fecal sample was transferred for pre-enrichment to buffered peptone water in the ratio of 1:9 and incubated for 24 ± 3 h at 37°C after that, 0.1 ml of the sample were selectively enriched in 10 ml of Rappaport Vassiliadis Soy Broth (RVS) and incubated aerobically at 41.5±1°C for 24 ± 3 h and then loop full of samples were platted out on xylose lysine deoxychlorate (XLD) medium incubated at 37°C for 20 to 24 h. Then *Salmonella* suspected colonies were examined for the presence of typical red colony with black center in XLD medium. Suspected colonies were cultured on nutrient agar and confirmed

by biochemical tests: TSI, Urease, Indole, lysine decarboxylase and Vogues Proskauer tests (Table 1). At this level, the genus of *Salmonella* was identified and suspected colonies were cultured on Biology Universal Growth (BUG) media for further species and subspecies confirmation.

For species and sub-species identification, OMNILOG (fully automated coated microplate based bacterial identification system) that is, GEN III microplate with protocol A method was used to test suspected colonies. A single colony grown on agar medium was selected and emulsified into 'inoculating fluid A' (IF A). According to the manufacturer's instructions, cell density of the bacterial inoculum was measured for a specified transmittance (90 to 98%) using a turbidimeter, as specified in the user guide. For each isolate, 100µl of the cell suspension was inoculated in to each of the 96 well coated microplate, using automatic multichannel pipette and incubated aerobically at 33°C for 22 h. The OMNILOG identification system automatically read each microplate and provides identification called species/ sub-species ID, and then the results were printed. The results were also read in the BIOLOG Micro Station reader after 22 h incubation outside GEN III incubator.

Antimicrobial Susceptibility Tests

The antibiotic susceptibility tests of the Salmonella isolates were performed according to the Clinical and Laboratory Standards Institute guidelines [14] by using the Kibry-Bauer disk diffusion test on Muller-Hinton agar (OXOID CM0337, England). Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube containing 5 ml of saline water and emulsified. The turbidity was measured using densitometry and adjusted to 0.5 McFarland. The sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller- Hinton agar plate within a sterile safety cabinet. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic discs with a known concentration of antimicrobials were placed and the plates were incubated for 24 hrs. at 37°C. Each isolate was tested for a series of six (6) antimicrobials. The following disks were used: Tetracycline (30µg), Gentamicin(10µg), Chloramphenicol(30µg), Polymixin B, Ampicillin (10µg), Sulphamethoxazole + trimethoprim (30µg) disks were used. The results were classified as resistance, intermediate, and susceptible after the zone of inhibition of bacterial growth is measured, hence a standardized table is given by the manufacturer for this classification [14]

Questionnaire survey

Questionnaires were administered to dairy owners to assess the general husbandry practices of calves in the farms. Generally, the questionnaire included all practices in the farm that were performed about, bedding, frequency of changing bed, colostrum feeding time and feeding up to weaning which affects the growth of calves.

Statistical analysis

The data generated from the study was arranged, coded, and entered into an Excel spreadsheet (Microsoft® office excels, 2007). The prevalence of Salmonella was calculated as a percentage of Salmonella culture-positive samples among the total number of samples examined. A Chi-square test was done by STATA version 13 to observe study the association between Salmonella isolates and risk factors. The significance level was set at 0.05 alpha value and 95% confidence level.

RESULTS

Laboratory examination of 116 fecal samples from diarrheic calves during the study period was done to isolate and identify *Salmonella* associated with calf diarrhea. The overall occurrence of *Salmonella* isolates were 8 (6.89%) *Salmonella* isolates which were identified using culture and biochemical tests were further processed for conformation and subspecies identification by using BIOLOG (semi-automatic machine). A total of 8 *Salmonella* isolates, consisting of two different subspecies were identified.

The subspecies identified during the study were *S. enterica* subspecies *enterica* and *S. enterica* subspecies *typhimurium*. *S. enteric* subspecies *enterica* constitutes 75% (6 isolates) and *S. enterica* subspecies *typhimurium* constitutes 25% (2 isolates). The *Salmonella* among calves of different age groups, sex, farm size, and times of colostrum feeding was determined. In this regard, the isolation rates of *Salmonella* decreased with the increasing age of calves. The difference in isolation rates of *Salmonella* in different age groups, body condition, times of colostrum feeding, bedding, frequency of changing bed was statistically significant (p=0.05).

All *Salmonella* suspected colonies were selected and cultured on nutrient agar media. Thus, *Salmonella* isolates were preliminarily characterized by IMViC tests: Indole, Methyl red, Voges- Proskauer, and Citrate utilization and TSI, Urea, Deoxylase Lysine and Motility test (Table 1).

Table 1: Biochemical tests performed on *Salmonella* isolates.

Indole	MR	VP	Citrate	Motility	Urease	Lysine	TSI	Isolated Bacteria	Biolog Result
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+
-	+	-	+	-	-	+	K/A/H2S+	*Salmonella*	+

In this study, the large farm size could be the cause for the higher *Salmonella* isolates. Calves housed in large herd size farms had the probability of being infected by *Salmonella* 18.3 times greater than as compared with small farm size (OR=18.3, P=0.010) (Table 5). Calves that were fed colostrum after 24 hours of birth were at a significantly higher risk of being infected with diarrhea due to *Salmonella* is 12.6 than those calves that were fed colostrum before six hours after birth (OR=12.6, p=0.023).

The result of this study shows that the salmonella isolate was higher in calves' pens which practiced changing the bedding material less frequently. This finding illustrates that the frequency of changing of bed <once/week were about 14.5 times more likely to be infected by *Salmonella* as compared with frequency of changing of bed >once/week (OR=14.5, P=0.016) (Table 6).

(MR) Methyl Red, (VP) Voges-Proskauer, TSI (Triple Sugar Iron) (+) Positive reaction, Negative reaction, (K/A/H2S+) Alkaline Slant and Acidic butt with Hydrogen Sulfide production.

All of the 8 isolates of *Salmonella* from diarrheic calves were subjected to a panel of 6 antimicrobials. The antimicrobial susceptibility profile of the isolates indicated that the isolates were 100% susceptible to sulphamethaxole + trimethoprimand Polymixin B, 87% to ampicillin, tetracycline and chloramphenicol and 75% to gentamycin. However, only one isolate showed mono drug (tetracycline) resistance (Table 2).

DISCUSSION

Salmonella infection in dairy cattle persists to be a major problem worldwide. Considerable economic losses were manifested through mortality and poor growth of infected animals as well as the risk of transmission to humans either through the food chain or direct animal contact. Hence, isolation, identification, and antibiotic sensitivity test from diarrheic calves were essential to control calves' mortality and morbidity in the farm.

In the present study, from a total of 116 diarrheic calves, 8 (6.89) % of the isolates tested positive for *Salmonella*. This finding was not in line with the report of Meganck *et al.* [15] (18.33%) and Fufa *et al.* [16] (43.75). However, this result was found to be agree with the report of Atnafu Regassa *et al.* [17] (6.2 %) (2020) and less than the finding of Tesfaye [18], (11.42) (2020). The variations in the *Salmonella* isolates found in the present study in Ethiopia as well as in other countries could possibly be due to variations in climatic conditions, management practices, including hygienic conditions, time of first colostrum feeding and housing system of the farms. Indicating that gaps in management includes inadequate nutrition, exposure to severe environment, insufficient attention to the new born calf, or a combination of these factors are often involved in scours outbreaks.

In this study, the large herd size could be the cause for the higher *Salmonella* isolates. Calves housed in large herd size farms had the probability of being infected by *Salmonella* 18.3 times greater than likely as compared with small farm size (OR=18.3, P=0.010) which is in agreement with previous reports [19-20]. This could be due to overcrowding of animals in the larger herds, especially those housed indoors, thus increasing animal to animal contact which enhances transmission of pathogens within the herd.

The present study compared calves of age 4-6 months had a 0.8 times higher probability of infected by *Salmonella* as compared with calves of age group 0-3 months (OR 08, p=0.023). This finding is similar to the report of Wondmu [21] that calves aged between 0-3 months were at a higher risk of diarrhea, particularly during the first week of life and the risk decreased with age increased **(**Table 3**)**.

This study identified statistically significant associations between calves' body condition and the occurrence of calves' diarrhea. Calves with good body condition had the probability of being infected by *Salmonella* 0.07 times greater as compared with calves of poor body condition after considering another variable constant (OR= 0.07, p=0.019) (Table 5). This indicates that the first six hours are the period in which maximum absorption of colostral immunoglobulin takes place. Therefore, delays in administration could lead to a lack of colostrum originated from maternal antibodies to protect calves from enteric pathogens.

Calves that were fed colostrum after 24 hours of birth were at a significantly higher risk of being infected with diarrhea due to *Salmonella* was 12.6 times greater than those calves that were fed colostrum before six hours after birth (OR=12.6, p=0.023).The difference in amount and relative occurrence of *Salmonella* isolate between the present and previous studies at different areas of the Ethiopia could be attributed to difference in risk factors that contribute to the occurrence of *Salmonella*. These are host related risk factors that include age, breed, the physiological state of the animals, feeding strategies, vaccination status [22]. Environment related risk factors such as hygienic and management practice, educational status, bedding, frequency of

Table 2: Antimicrobial sensitivity pattern of Salmonella isolates to tested drugs.

Antimicrobial agents	No. of S. (%)	No. of I. (%)	No. of R. (%)
TE	7 (87.5%)		1 (12.5%)
GM	6 (75%)	1(12.5%)	1 (12.5%)
C	7 (87.5%)		1 (12.5%)
PB	8 (100%)		0
AM	7 (87.5)	1(12.5%)	
SXT	8 (100%)		

S: Sensitive; I: Intermediate; R: Resistant; TE: Tetracycline; GM: Gentamycin; C: Chloramphenicol; PB: Polymixin B; AM: Ampicillin; SXT: Sulphamethaxole+trimethoprim

Table 3: Prevalence of *Salmonell*a occurrence with host related factors.

Variables	Categories	No. of samples Examined	No. of positive (%)	Chi- square	P-Value
Flock size	Small	66	1 (0.9%)	10.964	0.004
	Medium	24	3 (2.6%)		
	Large	18	4 (3.4%)		
Sex	Female	40	7 (6%)	0.077	
	Male	68	1 (0.9%)		0.781
Age in month	0-3 month	76	6 (5.2%)	7.869	
	4-6 month	32	2 (1.7%)		0.005
Breed	Local	38	2 (1.7%)	0.342	
	Cross	70	6 (5.2%)		0.559
Types of diarrhea	Watery	45	3 (2.6%)	2.547	
	Bloody	28	3 (2.6%)		
	Mucoid	35	2 (1.7%)		0.280
Body Condition	Poor	45	3 (2.6%)	10.807	
	Medium	28	4 (3.4%)		
	Large	35	1(0.9%)		0.004
Colostrum feeding	<6hrs	44	1(0.9%)	12.872	
	6-12hrs	43	1(0.9%)		
	>24hrs	21	6(5.2%)		0.002

Table 4: Prevalence of *Salmonell*a occurrence with environmental-related factors.

Variables	Categories	No. of samples examined	No. of positive (%)	Chi-square	P-Value
Educational status	Educated	50	3(2.6%)	0.232	
	Un educated	58	5(4.3%)		0.630
Bedding	Absent	71	1(0.9%)		
	Present	37	7(6%)	8.967	0.003
Frequency of changing bed	Once week	53	1(0.9%)	12.177	
	> Once week	33	1(0.9%)		
	< Once week	22	6(5.2)		0.002
Frequency of feeding	Once/day	44	3(2.6%)	0.226	
	Twice/day	32	3(2.6%)		
	Three time/day	32	2(1.7%)		0.893
Feeding up to weaning	Mikl replacer	78	3(2.6%)	4.262	
	Milk	30	5(4.3%)		0.039

feeding and frequency of changing bed play role for *Salmonella* occurrence [20], **(**Table 4**)**.

The *Salmonella* isolates were also higher in calves' pens which practiced changing the bedding material less frequently. This finding revealed that the frequency of changing of bed <once/week were about 14.5 times more likely to be infected by *Salmonella* as compared with frequency of changing of bed >once/week after describing other variables constant (OR=14.5, P=0.016), which was in consistent with report of Wondmu [23] (Table 6).

Therefore, adequate and fundamental attention should be given to the management of calf by ensuring colostrum feeding with in the first few hours' parturition in case of difficultly in feeding, and calf should be hand-fed with feeders. Overcrowding of calves was one of the majors is a risk factor and should be prevented as much as possible. The calf kept in-housed pens should be housed individually in clean pens. Farms practicing an intensive system of farming should pay ultimate attention to hygiene since they are at greater risk of the infection.

The revelation and disperse of antimicrobial resistance are an important concern in public health, animal health, and food safety. Therefore, antimicrobial susceptibility test was performed for all of the eight (8) bacterial isolates. In this study, most of the bacterial isolates were susceptible to sulphamethaxole +

Table 5: Prevalence of *Salmonella* occurrence with host-related factors using univariate logistic regression.

Variables	Categories	No. of samples Examined	No. of positive (%)	Crude OR [95% CI]	p-value
Flock size	Small	66	1 (0.9%)		
	Medium	24	3 (2.6%)	5.5[0.47-10.3]	0.172
	Large	18	4 (3.4%)	18.3[2.01-167]	0.010*
Sex	Female	40	7(6%)		
	Male	68	1(0.9%)	0.1[0.01-0 .70]	0.782
Age in month	0-3 month	76	6(5.2%)		
	4-6 month	32	2(1.7%)	0.8[0.15- 4.1]	0.023*
Breed	Local	38	2(1.7%)		
	Cross	70	6(5.2%)	1.6[0.31-8.5]	0.562
Types of diarrhea	Watery	45	3(2.6%)		
	Bloody	28	4(3.4%)	2.14[0.44- 10]	0.341
	Mucoid	35	2(1.7%)	0.4[0.04- 4.3]	0.471
Body Condition	Poor	45	3(2.6%)		
	Medium	28	4(3.4%)	0.15[.02- 0.9]	0.037
	Large	35	1(0.9%)	0.07[0.01- 0.65]	0.019*
Colostrum feeding	<6hrs	44	1(0.9%)		
	6-12hrs	43	1(0.9%)	1.02[0.06- 16.9]	0.987
	>24hrs	21	6(5.2%)	12.6[1.42-111]	0.023*

OR: odds Ratio; *: Statistically Significant

Table 6: Prevalence of *Salmonella* occurrence with environmental-related factors using univariate logistic regression.

Variables	Categories	No. of samples Examined	No. of positive (%)	Crude OR [95% CI]	p-value
Educational status	Educated	50	3(2.6%)		
	Un educated	58	5(4.3%)	1.4[0.32-6.31]	0.631
Bedding	Absent	71	1(0.9%)		
	Present	37	7(6%)	13.4[1.59- 113]	0.017*
Frequency of changing bed	Once week	53	1(0.9%)		
	> Once week	33	1(0.9%)	1.6[0.09- 26.6]	0.741
	< Once week	22	6(5.2)	14.5[1.64- 127.2]	0.016*
Frequency of feeding	Once/day	44	3(2.6%)		
	Twice/day	32	3(2.6%)	1.4[0.26-7.25]	0.708
	Three time/day	32	2(1.7%)	0.9[0.14-5.8]	0.926
Feeding up to weaning	Milk replacer	78	3(2.6%)		
	Milk	30	5(4.3%)	4.3[0.97-19.3]	0.054*

OR: odds Ratio; *: Statistically Significant v

trimethoprim and Polymixin B, ampicillin, chloramphenicol as well as gentamycin but, only one isolate showed mono drug tetracycline resistance.

CONCLUSIONS AND RECOMMENDATIONS

- Calves' diarrhea is a major cause of productivity and economic loss to cattle producers in various parts of the world. Calves' diarrhea causes significant economic loss in the dairy industry due to the treatment costs, labor costs, poor growth performance, high mortality, and morbidity.

While the present study, isolation and identification of Salmonella from diarrheic calves were conducted in Sebeta town Dairy farms. Out of 116 samples, 6.89 % tested positive for Salmonella. Salmonella occurrence was found to be significantly associatiated with on various potential risk factors such as; farm size, age group, body conditions of calves, time of first colostrum's feeding, the frequency of changing the bedding, and feeding up to weaning. The antimicrobial susceptibility pattern of *Salmonella* isolates showed 100% susceptibility to

Polymixin B, and Sulphamethoxazole + trimethoprim. Therefore, based on the above conclusion, the following points are forwarded as recommeGood animal husbandry and management activities should be practiced at various levels targeting to the above predisposing factors to minimize Salmonella infections.

- Awareness creation for farm owners about the appropriate timing of colostrum feeding and frequently changing the bedding practice.
- Use of effective drugs and treatment of animals by professionals to minimize the impact of infection.
- Regular antimicrobial susceptibility tests against Salmonella should be conducted to monitor the emergence of new MDR strains.

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