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Isolation, identification and antimicrobial Susceptibility patterns of *Salmonella* isolates from diarrheic calves in Bahir Dar city dairy farms, North West Ethiopia

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Abstract
Calf diarrhea is a major economic concern in bovine industry all around the world. Thus, this study was conducted to Isolation, Identification and Antimicrobial Susceptibility Patterns of Salmonella Isolates from Diarrheic Calves in Bahir Dar City Dairy Farms. A cross-sectional study was conducted in dairy farms in Bahir Dar city from November 2018- May 2019 with a purposive sampling method the objective of isolating and identifying Salmonella isolates from diarrheic calves. Potential risk factors associated with calf diarrhea and antimicrobial susceptibility patterns of few selected antibiotics were also performed. Fecal samples were directly collected from the rectum of diarrheic calves in order to identify Salmonella based on their cultural and biochemical characteristics. A total of 105 fecal samples were examined for the isolation of Salmonella, of which 12 (11.42%) were positive. Calves age (OR=0.28, P=0.028), body condition (OR=0.30, P=0.034), farm size (OR=0.27, P=0.047), calves kept in their pens with frequency of change of bedding material (OR=10, P=0.01), educational status of calves taker (OR=7.07, P=0.008), time of first colostrum feeding (OR=24, P=0.006), daily milk replacer given (OR=0.12, P=0.02) and feeding up to weaning (OR=0.13, P=0.01) were closely linked with Salmonella infection. Amongst all the antibiotics that were tested, Salmonella was found to be susceptible to ciprofloxacin, chloramphinicol and tetracycline (100%, 83.3%, and 75%) respectively whereas all the isolates showed resistance (100%) to ampicillin, cefoxitin and sulfonamides. In conclusion, Salmonella could be considered as a potential causative agent of diarrhea in calves. Therefore, improved management practices and appropriate antibiotic treatment of diarrheic calves should be employed to prevent the spread of Salmonella infection in healthy animals.

Keywords: Antimicrobial susceptibility, Bahir Dar city, diarrheic calves, risk factors, salmonella

Introduction
Newly born calves represent an important source of animal production for either meat or breeding worldwide. 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 [8]. Calves are at greatest risk of developing diarrhea within the first month of life, and the incidence of diarrhea decreases with age [26].
Several enteropathogens are implicated in neonatal calf diarrhea, and their relative prevalence varies geographically but the most common prevalent infections in most areas are *Escherichia coli*, Rotavirus, and Corona virus, *Clostridium perfringens*, *Salmonella*, and Cryptosporidium. Causes of neonatal calf diarrhea are commonly associated with more than one of these agents, and the causes of most outbreaks are usually multifactorial [16],[4],[8]. Among these organism *Salmonella* is the main cause for the calves diarrhea as white scour diarrhea. *Salmonella* is gram negative, rod shaped, flagellated, and motile (except *Salmonella Pullorum* and *Salmonella Gallinarum*) oxidaze negative, facultative anaerobe and is classified under the family Enterobacteriaceae [39].
Salmonella is one of the microorganisms of the lower gastrointestinal tract of mammals, including humans, and usually exists as a harmless communal. However, there also exist many pathogenic strains of *Salmonella* that can cause variety of diseases in both humans and animals [14].

Salmonellosis is a common bovine disease [30]. The genus *Salmonella* is divided into two species (*S. enterica* and *S. bongori*) and more than 2500 serovars. Serovars can be host adapted, such as bovine host adapted *S.* Dublin, or not host-adapted, such as *S.* Typhimurium. The pathophysiology of diarrhea associated with *Salmonella* infection is complex and involves inflammation and necrosis [30, 31].

Salmonellosis is a calf-hood disease that has major impact on the economic viability of cattle operations [23] which is documented by an increase in incidence especially that caused by *S.* Typhimurium in calves associated with the development of intensive rearing system. Although *Salmonella* infections may occur at any age in cattle, the associated clinical symptoms are more severe in calves from the first 2 weeks - 3 months of their life [3]. Substantial economic losses were manifested through mortality and poor growth of infected animals as well as the hazard of transmission to humans either through the food chain or direct animal contact [29].

Calves are infected by 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 [21].

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 [6]. Persistence of infection is an important epidemiologic feature of salmonellosis and can be related to serotypes with which animal is infected [20, 41].

Different studies conducted in Ethiopia revealed fragmented substantial isolation rate as well as antimicrobial susceptibility of *Salmonella* in veterinary and public health setups [17]. However, reports from coinciding study on apparently healthy animals at farm level, personnel and equipment used in the farms is limited especially in the current study area. Investigation of the prevalence and antimicrobial resistance of *Salmonella* from cattle and personal working in dairy farms is of paramount importance to design methods to minimize the possible transmission of *Salmonella* between humans and cattle. Moreover, it is also important in combating the emergence of antibiotic resistant strains of *Salmonella* [1].

Statement of the Problem

Salmonellosis is a widespread disease and can be found on a large number of dairy producers. It could be lead to losses from: milk production decline, death in any age group of livestock, abortions, treatment costs, losses from antibiotic contaminated milk, increased culling, increased cost due to delayed culling while antibiotic residues clear, increased labor for management of sick animals, reduced feed efficiency and the inability to sell animals originating from an infected herd. *Salmonella* infection in Bahir Dar City dairy farms is also a significant public health risk to farm families and employees. This disease has serious economic, animal health and public health implications. Hence, research on isolation, identification and antimicrobial susceptibility patterns of *Salmonella* in diarrheic calves in dairy farms plays a great role. 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.

Objective of the Study

General Objective

- To isolate and determine the antimicrobial susceptibility patterns of *Salmonella* from diarrheic calves in Bahir Dar City Dairy Farms.

Specific Objectives

- To isolate and identify *Salmonella* from cases of calf diarrhea in the study area.
- To determine antimicrobial susceptibility patterns of *Salmonella* isolates.

Research Questions

1. What is the extent of occurrence of *Salmonella* in diarrheic calves in Bahir Dar City dairy farms?
2. What are the antimicrobial susceptibility patterns of *Salmonella* in diarrheic calves?
3. What are the risk factors of *Salmonella* associated with diarrheic calves?

Study designs and methods

Description of the Study area

The study was conducted from November 2018 - May 2019 in dairy farms in Bahir Dar city which is located 565 kms away from North-West Ethiopia Addis Ababa, and having a latitude and longitude of 11°36'-12°15'N and 37°23'-38°20'E respectively. The City has an elevation of 1840 m above sea level [12].

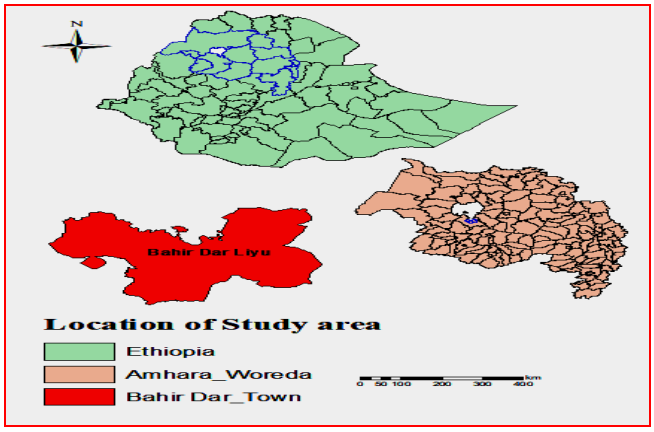

Fig 1: Map of Study Area Bahir Dar City [5].

Study Animal

Animals that were included in this study were cross and local breeds of dairy 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, dehydration, and reduced suckle reflex), and defecate pasty–watery feces. All diarrheic calves in dairy farms of Bahir dar city were incorporated for sampling during the study period in the study for a period. Fifty-four small sized and five large sized dairy farms were involved in the study period and classified as small (< 20 head of dairy cow) and large (>20 head of dairy cow) [33]. Ages of diarrheic calves were categorized into two age groups: 0-3-month age and 4-6-month age, according to [22]. 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].

Study design and sampling methodology

A cross sectional study was conducted from November 2018 - May 2019 with a purposive sampling method, i.e., samples was collected from calves that show clinical signs of diarrhea during the study period. The sample size was determined based on availability of clinical case (diarrheic calves). Approximately 25g of fecal sample was collected from non-treated diarrheic calves directly from the rectum by using disposable wrist length gloves and was transferred immediately to sterile universal bottles. Then samples were transported in ice box to the Veterinary Microbiology Laboratory, University of Gondar, for processing. Fecal samples were stored at +4 °C until the time of processing.

Isolation and Identification of *Salmonella*

The 25 gram of fecal sample was enriched in 225ml of Buffered peptone water (BPW) (Oxoid, England) and incubated at 37°C for 24 h and then transferred in to 1ml of pre-enrichment before inoculation to 10ml tetrathionate broth (Muller-Kaufmannn). In addition, 0.1 ml of the pre-enrichment media containing bacteria were transferred into 10 ml Rappaport Vassiliadis soy peptone (RVS) broth and incubated at 37°C for 24h. After 24h, a loopful from the selective enrichment broth were inoculated onto xylose lysine deoxycholate (XLD) (Oxoid) agar and incubated at 37°C for (18 - 24h). Characteristic *Salmonella* colonies from examined samples, showed a slightly transparent zone of reddish colour with a black center. The purified cultures of *Salmonella* were stored at +4°C temporarily as nutrient broth cultures for further identification by biochemical tests. Identification of suspected *Salmonella* colonies was conducted following standard bacteriological procedures. Thus, *Salmonella* isolates were preliminarily characterized by IMViC tests: indole, methyl red, Voges- Proskauer, and citrate utilization. The isolates which exhibited the IMViC pattern of -, +, −, +, respectively, catalase positive and TSI test (K/AG or H_2S+) were presumed as *Salmonella* isolates [32].

Antimicrobial Susceptibility Tests

The antimicrobial susceptibility testing of the *Salmonella* isolates was performed using a panel of six antimicrobial discs: ampicillin (10 µg), sulfonamides (300 µg), chloramphenicol (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg) and cefoxitin (30 µg) (Oxoid Ltd, England) using Kirby-Bauer disk diffusion test according to the Clinical and Laboratory Standard Institute guideline [9].

The inoculum was prepared by making a direct sterile saline water suspension of isolated colonies selected from a 24hour agar plate (nutrient agar). The suspension was adjusted to match with 0.5 McFarland turbidity standard using sterile saline water and a vortex mixer. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculums and was swabbed uniformly over the surface of Muller Hinton agar plate. The plates were held at room temperature for 15 min to allow drying. Then Antibiotic discs with known concentration of antimicrobials were placed on the cultured Muller Hinton agar plate of appropriate distance of each disc with flamed forceps, inverted and incubated with agar side up for 18-20 hours at 37ºC. Following incubation, the diameter of zone of inhibition was measured with an electronic digital caliper to the nearest millimeter by holding the plate a few inches above a black nonreflecting surface. For the susceptibility testing, interpretation of the results was depending on categorization of isolates into susceptible, intermediate or resistant according to CLSI guidelines [9].

Questionnaire survey

Questionnaires were administered to dairy owners to assess the general husbandry practices of calves. Generally, the questionnaire included all practices in the farm that were performed about calves health care, hygiene, and health problem, colostrum feeding time, duration of diarrhea that affect the growth of calves Appendix 5.

Data collection, management and analysis

Data describing the diarrheagenic conditions suggestive of *Salmonella* infection observed on calves along with age, breed, sex, farm size, body condition and a host risk factor and environmental risk factors of a questionnaire was classified, filtered, and coded using Microsoft Excel (2007).Then data was exported to STATA version 12 (STATA Corporation, College Station, TX) for statistical analysis. Descriptive statistics was performed to look in to the data pattern and then odds ratio of logistic regression analysis was used to measure associations between *Salmonella* isolates and risk factors such as age, breed, sex, farm size, body conditions, associations were reported as statistically significant if (P <0.05).

Results

Isolation and identification of *Salmonella*

For isolation of *Salmonella*, xylose lysine deoxycholate (XLD) (Oxoid) agar were used as selective plating media and the following biochemical tests (IMViC tests: indole, methyl red, Voges- Proskauer, citrate, catalase and TSI) were also used for identification of *Salmonella*. One hundred five (105) fecal samples were initially screened on XLD agar and after incubation at 37°C colonies that showed a slightly transparent zone of reddish colour with black centers were selected for further biochemical tests. Among the total 105 fecal samples of diarrheic calves during the study, 12 isolates (11.42%) were found to be positive for *Salmonella*. Calves of 4-6 months age had a 0.28 times less likely probability of infected by *Salmonella* as compared with calves of age group 0-3 months (OR 0.28, P=0.028). Calves housed in small sized farms had the probability of infected by *Salmonella* 0.27 times less likely as compared with large farm size (OR=0.27, P=0.047). Calves with good body condition had the probability of being infected by *Salmonella* 0.30 times less likely as compared with calves of poor body condition after considering another variable constant (OR= 0.30, P=0.034). The results are summarized in Table 1.

Table 1: Association of Salmonella isolates occurrence with various factors

Variables	Category	N No.	*Salmonella* +Ve (%)	OR	p-value
Age (month)	0-3 4-6	67 38	8(7.61) 4(3.81)	0.28	- 0.028*
Breed	Cross Local	59 46	10(9.52) 2(1.9)	0.22	- 0.061
Sex	Male	65	4(3.8)	1.26	-

	Female	40	8(7.61)		0.719
Farm size	Large Small	41 64	8(7.61) 4(3.81)	0.27	- 0.047*
Body conditions	Poor Medium Good	35 58 12	8(7.67) 3(2.85) 1(0.95)	0.18 0.30	- 0.045* 0.034*

Key: *= statistically significant, OR=odds ratio, No. +Ve = number of positive calves, No= number of cases

Results of Biochemical tests on *Salmonella* Isolates

A diverse range of biochemical tests are known for the identification and differentiation of *Salmonella*. A conventional biochemical test is often used to identify microorganisms; the results were observed by color change and confirmation was made based on the reaction of an enzyme with a specific substrate. *Salmonella* was identified using the following biochemical (IMViC tests: indole, methyl red, Voges- Proskauer, and citrate utilization with the result -, +, -, + respectively). Further biochemical tests on the isolates yielded the following results: catalase positive, alkaline slant/acidic butt with gas production/ hydrogen sulfide (H_2S) with TSI test (K/AG or K/A/H_2S+) Table 4.2.

Table 2: Results of various biochemical tests performed on Salmonella isolates

Samples	Indole	Citrate	MR	VP	Catalase	Urease	TSI	Isolated Bacteria
A04	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A09	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A12	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A16	-	+	+	-	+	-	K/AG	*Salmonella*
A23	-	+	+	-	+	-	K/AG	*Salmonella*
A32	-	+	+	-	+	-	K/AG	*Salmonella*
A36	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A54	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A61	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A73	-	+	+	-	+	-	K/AG	*Salmonella*
A81	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*
A82	-	+	+	-	+	-	K/A/H_2S+	*Salmonella*

Key: (MR) Methyl Red, (VP Voges-Proskauer, (+) Positive reaction, (-) Negative reaction, (K/AG) Alkaline Slant and Acidic butt with gas production, (K/A /H_2S+) Alkaline Slant and Acidic butt with Hydrogen Sulfide production

Antimicrobial Susceptibility Profiles of *Salmonella* isolates

Antimicrobial susceptibility testing was performed for all *Salmonella* isolates from diarrheic calves using Six (6) antimicrobial impregnated discs (Oxoid, England). *Salmonella* isolates showed the highest resistance (100%) against ampicillin, cefoxitin and sulfonamides Table 4.3. However, the isolates were found to be susceptible to ciprofloxacin, chloramphenicol and tetracycline (100 %, 83.3% and 75%) respectively. The summarized susceptibility patterns of *Salmonella* species (N = 12) the antibiotics that were tested are shown in appendix 4.

Table 3: Antimicrobial susceptibility pattern of Salmonella isolates

Antibiotic agent	Disk potency (µg)	Diameter of the inhibition zones of *Salmonella* according to CLSI (mm)			Number of isolates (n=12)		
		S	I	R	S (%)	I (%)	R (%)
AMP	10	≥17	14-16	≤ 13	0(0.0)	0(0.0)	12(100)
S3	300	≥17	13-16	≤12	0(0.0)	0(0.0)	12(100)
CN	30	≥18	13-17	≤12	10(83.3)	0(0.0)	2(16.6)
TC	30	≥15	12-14	≤11	9(75)	0(0.0)	3(25)
CPR	5	≥21	16-20	≤15	12(100)	0(0.0)	0(0.0)
FOX	30	≥22	--	≤21	0(0.0)	0(0.0)	12(100)

Key: %=Percent, S=Sensitive, I=Intermediate, R=Resistant, AMP=Ampcillin S3=Sulfonamides, CN=Chloramphenicol, TC=Tetracycline, CPR=Ciprofloxacin and FOX=cefoxitin

Multi Drug-resistance pattern (MDR) of Salmonella isolates

All *Salmonella* isolates developed MDR to different antibiotics. The most frequent MDR pattern consisting of three drugs were found against ampicillin, cefoxitin and sulfonamides with a resistance of 100%. Three isolates were found to be resistant to four antimicrobial drugs namely ampicillin, cefoxitin, sulfonamides and tetracycline, while two other isolates were found to be resistant to ampicillin, cefoxitin, sulfonamides and chloramphenicol Table 4.4

Table 4: Multidrug resistance (MDR) distribution among Salmonella isolates

Number of antimicrobial resistance	Antimicrobial resistance patterns (number of isolates)	Number of isolates (%)
Three	AMP,FOX,S3 (12)	12(100)
Four	AMP,FOX,S3,TC (3)	3(25)
	AMP,FOX,S3,CN (2)	2(16.67)

Keys: AMP=Ampicillin, FOX=Cefoxitin, S3=Sulfonamides compound, CPR= Ciprofloxacin, CN=Chloramphenicol, TC=Tetracycline

Questionnaire results

Potential risk factors from the questionnaire were categorized as: (a) host risk factors such as age group of affected calves, time of first colostrums feeding, breed, sex, type of diarrhea and (b) environmental risk factors such as, method of colostrums feeding, feeding up to weaning, daily milk replacer given, frequency of changing of bedding, educational status of calves taker, treatment of sick animal, experience as calves taker, method of availing veterinary services, frequency of feeding and duration of feeding.

Most large farms had calving facility (separate calving pen) and were aware of the immunological importance of colostrums (All the farms had the knowledge of benefits of colostrums feeding over ordinary milk). However, a deliberate effort to feed calves with colostrum at the right time (<6 hours) was practiced only in the large sized dairy farms. 0.95% of the calves were positive for *Salmonella* which were fed colostrums before 6 hours after birth, 2.85% calves were positive for *Salmonella* which fed colostrums between 6 and 12 hours after birth, and 7.61% calves were positive for *Salmonella* which were fed colostrums after 12 hours of birth. The associations of various host risk factors in the affected age group of calves was found to be 4-6 months of age which was 0.21 times less likely to be infected by *Salmonella* compared to age of 0-3 months (OR=0.21, P=0.025).

Calves that obtained colostrum > 12 hours 24 times more likely a probability of infected by *Salmonella* as compared with calves fed colostrum within 6 hours by considering other variable constant (OR=24, P=0.006). Results of various host risk factors which were included in questionnaire are summarized in Table 4.5.1.

Table 5: Results of various host risk factors included in questionnaire

Factors	Category	Collected Questionnaire	Total Positive n (%)	OR	P-Value
Affected age group	0-3 month	28	10(9.52%)	-	
	4-6 month	31	2 (1.9%)	0.21	0.025*
Affected Sex	Female	33	8(7.61%)	-	
	Male	26	4 (3.8%)	1.76	0.40
Breed	Cross	41	10(9.52%)	-	
	Local	18	2 (1.9%)	0.38	0.25
Type of Diarrhea	Watery	16	3 (2.85%)	-	
	Bloody	22	5(4.76%)	1.27	0.76
	Mixed	13	5(4.76%)	0.78	0.81
	Mucoid	8	2 (1.9%)	1.4	0.72
Time of first colostrum feeding	<6 hour	22	1(0.95%)	-	
	6-12hour	22	3(2.85%)	3.31	0.31
	>12 hour	15	8(7.61%)	24	0.006*

Key: *= statistically significant and OR=odds ratio

Results of various environmental risk factors included in questionnaire are summarized in Table 4.5.2. Calves pen with the frequency of changing of bedding <once/week were about 10 times more likely infected by *Salmonella* as compared with frequency of changing of bedding >once/week after describing other variables constant (OR=10, P=0.01).

Calves managed by uneducated calves takers were 7.07 times more likely to be infected by *Salmonella* compared to calves managed by educated calves taker (OR=7.07, P=0.008).

Calves that obtained daily milk replacer as three liter/day were about 0.12 times less likely to be infected by *Salmonella* as compared with those that obtained one liter milk replacer/day after describing other variable constant (OR=0.12, P=0.02). Calves fed with milk up to weaning only were about 0.13 times more likely to be infected by *Salmonella* as compared to calves that obtained feed milk replacer up to weaning (OR=0.13, P=0.01).

Table 6: Results of various environmental risk factors included in questionnaire

Factors	Category	Collected Questionnaire	Total Positive n (%)	OR	P-Value
Method of feeding	Suckling	28	4 (3.8%)	-	
	Hand feeding	31	8 (7.61%)	2.08	0.27
Bedding	Present	29	8(7.61%)	-	
	Absent	30	4(3.8%)	0.40	0.18
Frequency of changing the bedding	>once/week	22	2(1.9%)	-	
	Once/week	25	4(3.8%)	1.9	0.48
	<once/week	12	6(5.71%)	10	0.01*
Treatment for sick calves	Antibiotic	33	4(3.8%)	-	
	Antihelmentic	26	8(7.61%)	3.22	0.08
Method of availing veterinary services	By taking the clinic	20	2(1.9%)	-	
	Calling Veterinarian	20	4(3.8%)	2.25	0.38
	Calling animal health Technician	19	6(5.71%)	4.15	0.11
Educational status of calves taker	Educated	36	3(2.85%)	-	
	Un educated	23	9(8.57%)	7.07	0.008*
Experience as calves taker	≤ 5 years	28	8(7.61%)	-	
	>5 years	31	4(3.8%)	0.37	0.14
Daily milk replacer given	One liter	17	7(6.66%)	-	
	Two liter	18	3(2.85%)	0.28	0.11

	Three liter	25	2(1.9%)	0.12	0.02*
Frequency of feeding	Once/day	20	7(6.66%)	-	
	Twice/day	27	3(2.85%)	0.23	0.05
	Three time/day	12	2(1.9%)	0.37	0.27
Duration of feeding	Day only	30	6(5.71%)	-	
	1-3 day	11	3(2.85%)	1.5	0.61
	>3day	18	3(2.85%)	0.8	0.77
Feeding up to weaning	Milk replacer	30	2(1.9%)	-	
	Milk	29	10(9.5%)	0.13	0.01*

Key: *= statistically significant and OR=odds ratio

Discussion

In the present study, from a total of 105 diarrheic calves, 11.42 % of the isolates tested positive for *Salmonella*. This finding were found to be in agreement with the report of Sunday [35] (13%) and Manickam [25] (18.33%). In the present study, the *Salmonella* isolated were lesser than results of Fufa Abunna [17] (43.75%). However, *Salmonella* isolates found in the present study was much higher than the reports of [36], [15]. 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. Charles [7] indicated 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. 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* 0.30 times less likely as compared with calves of poor body condition after considering other variable constant (OR= 0.30, P=0.034).Thus calves that were fed colostrum after six hours of birth were at a significantly higher risk of being infected with diarrhea due to *Salmonella* than those calves that were fed colostrum before six hours after birth. Calves that obtained colostrum after 12 hours of birth were 24 times more likely to be infected by *Salmonella* as compared with calves fed colostrum within 6 hours after birth by considering other variable constant (OR=24, P=0.006). This finding is agreement with that of [24], [40] who found that each hour of delay in colostrum ingestion in the first 12 hours of age increased the probability of a calf becoming ill by 10%. Colostral immunoglobulin (IgG) is absorbed within six hours and declines sharply thereafter. This indicated that in the first six hours, a maximum absorption of colostral immunoglobulin occurs. Therefore, delays in administration could lead to deficiency of colostrum originating maternal antibodies to protect calves from enteric pathogens.

The present study compared calves of age 4-6 months had a 0.28 times less likely probability of infected by *Salmonella* as compared with calves of age group 0-3 months (OR 0.28, P=0.028). This finding is similar to the report of [43] 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. This was well explained by Godden [18], Mellor and Stafferd [28], who reported that the structure of the bovine placenta impedes easy acquision of immunoglobulins by unborn calves during pregnancy and therefore calves are born without circulating protective antibodies, therefore they are more susceptible to various pathogens.

Herd size with a marked increase in population density commonly resulted in an increase in the incidence of infectious diseases and mortality [27]. The infections of calves with *Salmonella* were significantly higher in larger herd sizes [34, 38, 27]. Herd size by itself did not have a biological effect on the calf health; rather, it could be a measurement of other factors such as time available in order to observe and care for calves. Another possible explanation for the apparent association between herd size and calf mortality could be that in the case of small herd sizes, sufficient time could elapse between successive births, which would reduce the concentration of infectious agents in the calf-rearing environment. In this study, the large herd size could be the cause for the higher *Salmonella* isolates. Calves housed in small sized farms had the probability of being infected by *Salmonella* 0.27 times less likely as compared with large farm size (OR=0.27, P=0.047) which is in agreement with previous reports [42, 13]. 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 *Salmonella* isolated were also higher in calves pens which practiced changing the bedding material less frequently. This finding that the frequency of changing of bedding <once/week were about 10 times more likely to be infected by *Salmonella* as compared with frequency of changing of bedding >once/week after describing other variables constant (OR=10, P=0.01), which is in agreement with report of Wondmu Temesgen [43] and Amoki [2].

Farm ownership and educational status of calves takers could play an important role for the calves welfare and health [44]. Calves managed by uneducated calves takers were 7.07 times more likely to be infected by *Salmonella* as compared to calves managed by educated calves taker (OR=7.07, P=0.008).

The emergence and dissemination of antimicrobial resistance is an important concern in public health, animal health, and food safety. Thus, antimicrobial susceptibility test was performed for all of the bacterial isolates. In this study, most of the bacterial isolates were susceptible to ciprofloxacin, chloramphenicol and tetracycline. In the present study, the resistance profile of ampicillin, cefoxitin and sulfonamides were (100%) higher than reported by Guesh Mulaw [19] who reported (94.4%) and (36.6%) for ampicillin and cefoxitin respectively. Manickam [25] reported (75%) resistance to ampicillin which is lower than found in this study. On the other hand, the sensitivity of *Salmonella* to tetracycline (75%) was higher than reports of Guesh Mulaw [19] (30.6%) and Manickam [25] (26%). However, the sensitivity of *Salmonella* isolates to chloramphenicol (83.3%), and ciprofloxacin (100%) agreed with the reports of Guesh Mulaw [19]. Multi drug resistances of three out of the twelve *Salmonella* isolates obtained in this study showed MDR to ampicillin, sulfonamides and cefoxitin (100%) which could be the consequence of indiscriminate use of antibacterial in clinical practice which agrees well with the finding of constable [10].

Conclusion 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. During the present study, isolation and identification of *Salmonella* from diarrheic calves were conducted in Bahir Dar City Dairy farms. Out of 105 samples, 11.42 % tested positive for *Salmonella*. *Salmonella* occurrence was found to be significantly associated with various potential risk factors such as; age group, farm size, body conditions of calves, time of first colustrums of feeding, feeding up to weaning, daily milk replacer given, the frequency of changing the bedding and also the educational status of calves taker. The antimicrobial susceptibility patterns of *Salmonella* isolates showed susceptibility to chlorampenicol, tetracycline and ciprofloxacin. All *Salmonella* isolates showed multi drug resistance to ampicillin, cefoxitin and sulfonamides.

Therefore, based on the above conclusion, the following points are forwarded as recommendations:

- Better animal husbandry and management activities should be practiced at various levels of farm management in order to minimize *Salmonella* infections and the resultant economic losses.
- Awareness creation for farm owners about the appropriate timing of colostrums feeding practice.
- Use of effective drugs and treatment of animals by professionals in order to minimize the impact of infection
- Regular antimicrobial susceptibility tests against *Salmonella* should be conducted in order to monitor emergence of new MDR strains
- Veterinarians should be advised to use ciprofloxacin, tetracycline and chloramphenicol in their farms against diarrheal diseases of *Salmonella*.
- All farm owners need to avail the services of animal health professionals
- *Salmonella* species and strains should be identified using further immunological and molecular techniques.

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