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Research Article

Calf Diarrhea, Mortality and Antimicrobial Susceptibility Profiles with Emphasis on *E coli* and Salmonella Isolates in Selected Dairy Farms of Harar, Ethiopia

Biruk Akalu*

Harari Regional Agricultural Bureau, Livestock Development and Fishery Directorate, Harar, Ethiopia

***Corresponding Author:** Biruk Akalu, Harari Regional Agricultural Bureau, Livestock Development and Fishery Directorate, Harar, Ethiopia

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Abstract

A longitudinal prospective observational study on calf diarrhea and mortality in dairy farms in Harar was carried out from February to July 2017 with the objective of determining the incidence of calf diarrhea and mortality; examining potential risk factors related to calf diarrhea and mortality; identification of *Escherichia coli* and *Salmonella* associated with calf diarrhea and to determine their antimicrobial susceptibility profiles. A total of 130 calves, from 30 dairy farms in Harar were included in the study. Each calf was regularly monitored for the presence of diarrhea up to an age of six months. Information on risk factors were collected during the regular visit to farms and from questionnaire survey conducted during the study. Fecal samples were also collected from diarrheic calves for the detection of *E. coli* and *Salmonella*. The overall incidence risk of calf diarrhea and mortality were 29.39% and 9.69%, respectively. Among the risk factors examined, age of calf (OD = 0.06, P = 0.026), parity of the dam (OD = 3.39, P = 0.043), barn ventilation status (OD = 0.30, P = 0.048) and barn drainage status (OD = 2.95, P = 0.049) were found significantly associated with the incidence of calf diarrhea. Age of the first colostrum ingestion was the only factor that was found significantly associated with risk of calf mortality due to diarrhea. *E. coli* (47.22%) and *Salmonella* (16.67%) were detected from diarrheic calves. Relatively *E coli* were found to be susceptible for chloramphenicol and gentamycin but developed resistance against ampicillin and tetracycline whereas *Salmonella* was found to be susceptible for chloramphenicol and developed resistance against three drugs; gentamycin, erythromycin and amoxicillin. The result clearly showed that calf diarrhea and mortality rates found in this study was much high and could affect the productivity of the dairy farms by decreasing the availability of replacement stock. It is therefore, awareness creation and implementation of improved calf management practices and strategic use of antimicrobial drug in the study area would significantly reduce calf diarrhea and mortality.

Keywords: Calves; Diarrhea; Mortality; Antimicrobial Susceptibility Test

Introduction

Calves are the future herd and keeping them in a fit and healthy condition makes an effect on a livestockfarms' efficiency, economy and production outputs in a country [1]. The productivity of cattle depends largely on their reproductive performance and the survival of calves but it is challenged by early calf morbidity and mortality [2,3].

Calf morbidity and mortality are problems of major concern in all countries where cattle are raised underdifferent husbandry practices and the problem is more acute in developing countries [4]. In different parts of Ethiopia the incidence of calf morbidity and mortality ranging from 58.4-62% and 7-30.7%, respectively [4-11].

The common causes of calf diseases and deaths in dairy farms are diarrhea and pneumonia [12,13]. Calfdiarrhea is one of the

most common diseases in young animals, causing huge economic and productivity losses to bovine industry worldwide since the future of any dairy production depends on the successful raising of calves and heifers for replacement [14]. In addition to economic losses, diarrhea in calves is very important because of public health implications as numerous infectious agents causing diarrhea in animals are zoonotic and have been associated with food- borne diseases [15]. In Ethiopia researcher reported diarrhea as the most frequent disease syndrome in dairy calves [10,11]. *E. coli* and *Salmonella* species have also been reported as the most commonly detected bacterial causative agents of calf diarrhea [4,5,11].

Different managemental and environmental factors were reported to affect significantly calf diarrhea andmortality, these include; colostrum feeding, time of colostrum feeding, volume of colostrum fed, housing, calving assistance, production system, herd size, season, cleanness of the calf house and presence of dystocia in the dam at the time of birth [8,9,16,17].

The age of the calf is also the most important factor for the occurrence of morbidity and mortality. Approximately 75% of mortality in dairyanimals less than one year of age occurs in the first month of their life [18].

Antimicrobial agents are considered popular to fight diarrhea in calves. Nevertheless, their wide spectrum of activity, the emergence of microbial tolerance of different antimicrobial agents has becomea well-known phenomenon, which represents a major concern [19]. Resistance to antimicrobial agents was frequently occurred in *Salmonella species* and *E. coli* particularly in pre-weaned dairy calves [20]. Study conducted in Ethiopia indicated *E coli* and *salmonella* isolated from carcass were found resistant to different antimicrobial drugs [21,22]. and antimicrobial susceptibility testing is very crucial in the selection of an appropriate antimicrobial agent for the treatment of diarrhea.

In Ethiopia, calf morbidity and mortality were ranked next to mastitis as the second biggest problem for dairy production [23]. However there is lack of information on calf diarrhea and mortality as well as antimicrobial susceptibility profile of major bacteria isolated from calf diarrhea. Therefore the present study was conducted with the objectives to determine the incidence of calf diarrhea and mortality; and antimicrobial susceptibility pattern on selected major bacterial isolates for the appropriate prevention, control and treatments of the case in Harar where a number of dairy farms are available.

Materials and Methods

Study area

The study was conducted in Harar which is 525 Km east of Addis Ababa, the capital city of Ethiopia. The region is situated at 41°59' 58''N and 9°24'10'' E. with annual average temperature is between 17.1°C-20.2° C and the average annual intensity of precipitation ranges between 750-1,000 mm [24]. Study sites were selected purposively based on their dairy potential. There are small and large sized dairy farms which were managed under intensive management system.

Study animals

The sampling units for the study were dairy calves of up to 6 months of age. Calves less than one months of age from dairy farms in Harar were constituted as the study population.

Study design

The study was longitudinal prospective observational study (A prospective single closed cohort study). The calves were identified individually and monitored from the beginning of February to July 2017. The questionnaire survey was also carried out during the study period.

Sampling technique and sample size

All calves less than one month of age from 30 different dairy farms located in Harar city and engaged in market oriented dairy production were selected for the study. Dairy production systems were intensive production system. The data was collected from the study unit on events associated with calf health problem on longitudinal base and surveyed for potential risk factors on questionnaire basedduring the study period. Preliminary survey was conducted in the study area to assess the availability of information on the study animal and, but even if the farm list in the city can be accessed from the city agriculture bureau difficult to have information especially in relation to study animal found in a particular dairy farm.

Cluster sampling method, which is applicable in the absence of sampling frame, was used. The sample size for one stage cluster sampling was determined by the formula given by Thrusfield [25].

$$g = \frac{1.96^2\{nV_c + P_{exp}(1 - P_{exp})\}}{nd^2},$$

where:

g = number of clusters to be sampled.

n = predicted average number of animals per cluster;P_{exp} = expected prevalence.

d = desired absolute precision;V_c = between-cluster variance.

For the purpose of sample size determination, the expected calf mortality of 9.3% (reported by Bekele., *et al.* [26], confidence level of 95% required absolute precision of 5% was used. In addition an assumptionwas made on the between cluster variance component. An indication of the between cluster variance component (V_c), which is the variation expected between clusters if all animals in the clusters were sampled, assumed to be very small with small size clustering (four calves under one months of age per cluster).

Accordingly, a total of 30 study dairy farms, small and large size dairy farms were used for this study. Four average number of study animal per farm were selected and totally 130 calves (under one monthsof age) were included in the study.

Longitudinal study

Monitoring of dairy farms for calf diarrhea and mortality was carried out for 6 months from the beginning of February to July 2017. For the purpose of this study, morbidity was defined as diarrhea characterized by passing of lose or watery feces with increased frequency, which could or could not be accompanied by other systemic signs like dehydration, decreased appetite or fever; and mortality as death of calves due to diarrhea.

Individual records were used to record genealogy of the calf, events surrounding the birth of the calf, routine management practices applied to the calf and incidence of diarrhea that were observed during themonitoring.

Study calve were regularly visited every two weeks intervals by the researchers. During the regular farmvisits clinical examination of calves was made for the presence of diarrhea. In addition, observation on different calf management aspects like cleanness of the calf house and feeding practices was assessed. Inaddition to scheduled visits, emergency visits were paid in response to calls from farms for calf health problems.

Cross-sectional study

First the objective of the research was briefed to the owners and asked to participate in the research on voluntary basis Structured and pretested questionnaire was administered to the selected dairy farmers by one visit interview. The questionnaire was designed to collect information on farm characteristic, calf management practices, pre-parturient care, feeding and housing, and previous history of the calf disease.

Sample collection

About ten grams of feces was collected from rectum of untreated diarrheic calves soon after onset of diarrhea in separate sterile container, labeled and kept at an ice cold condition and transported to Microbiology laboratory, College of Veterinary Medicine, Haramaya University. The samples were collected with the intention of detecting *E. coli* and *Salmonella.*

Detection of *salmonella*

Isolation and identification of *Salmonella* was carried out based on procedures made available by international organization for standardization. About 5-10% of the quantity of feces will be inoculated into selenite cycitene (Merck, Germany) and 1% into Rappaport Vassiliadis (Sifini, Germany) broths which was incubated at 37°C and 42°C for 24 hours, respectively. From selective enrichments, subculture was made at 24 hours to selective plate media BPLS (Sifin, Germany) and XLD (Sifin, Germany) and incubated at 37°C for 24- 48 hours. Suspicious colonies were further subcultured in nutrient media (Oxoid, England) for biochemical tests. The suspected pure colonies from nutrient agar were inoculated in TSI slant (Merck, Germany), citrate slant (Difico, USA), lysine decarboxylase broth (Difico, USA) and urea broths (Merck, Germany). Red (alkaline) slant, yellow (acid) butt, H_2S positive/negative in TSI and lysine positive and urease negative was identified as *Salmonella*.

Detection of *E. coli*

Fecal samples was inoculated onto Eosin Methylene Blue (EMB) agar medium, which selectively growsmembers of the *Enterobacteriaceae* and permit differentiation of enteric bacteria on the basis of morphology, and incubated at 37°C overnight. Colonies showing characteristic metallic sheen on (EMB)agar was then picked up and considered as presumptive *E. coli.* The selected colonies were inoculated further on to blood and MacConkey agars. From each MacConkey agar plate both lactose fermenting and non-fermenting colonies was stored temporarily as nutrient broth cultures for further identification by biochemical tests. All the isolates was stained by gram stain to determine the cell morphology, gram reaction and purity of the isolates under the oil immersion lens (x100). Identification of suspected *E. coli* colonies was conducted following standard bacteriological procedures described in Quinn., *et al.* [27]. Thus, *E. coli* isolates was characterized preliminarily by biochemical IMViC tests, viz. indole, methyl red, Voges-Proskauer and citrate utilization. The isolates which exhibited IMViC pattern of (+ +- -) respectively was presumed as *E. coli* isolates.

Antimicrobial susceptibility testing

Antibiotic susceptibility was tested for E coli and Salmonella isolates by the disc diffusion techniques according to Clinical and Laboratory Standard Institute (CLSI) guidelines [28]. The pure culture colony suspension of the isolate was made using sterile physi-

ological saline and adjusted to 0.5 McFarland standards. Muller Hinton agar plate was swabbed with the suspension using sterile cotton swap and the antibiotic discs was placed over the agar and left for 30 minutes for diffusion of the antibiotics in the disc. The plates was inverted upside down and incubated at 37°C for 18 to 24 hours. The findings of antibiotic resistance testing were recorded as susceptible, intermediate and resistant according to Clinical and Laboratory Standards Institute break points [29]. Each isolates was tested against commonly used antimicrobials (chloramphenicol (30µ), amoxicillin (10µ), gentamycin (10µ), erythromycin (5µ) ampicillin (10µ) and tetracycline (30µ)

Data analysis

Incident density (I) was calculated by dividing the number of new cases interest occurred in the study animals during the follow up period to the sum of the length of time at risk of developing disease. The cumulative incidence (CI) was calculated by dividing the number of individual that become diseased during the study period to the number of healthy individual in the population at the beginning thatperiod.

Associations between risk factors (explanatory variable) and outcome variable (status variable) were done by logistic regression analyses. First individual risk factor was screened in terms of their simple (crude) association with an outcome variable by univariable logistic regression analyses. Those variablessignificantly associated with the outcome variable with $p < 0.25$ in univariable analysis were selected for multivariable analysis, a model were fitted for each outcome (status) variable stepwise backwardelimination of non-significant variables ($p < 0.25$). STATA was used to run logistic regression.

Results

Descriptive statistics

All the farms included in this study they kept dairy animals under intensive diary production system. Allfarms serve their cows using artificial insemination and raise their own replacement stock and none had calving facility. The knowledge of immunological importance of colostrums was present in all farms,but only 10% [3] of farms feeding calves with colostrums at the right time practiced.

All study farms fed whole milk for calves two times per day and the amount of milk fed per head of calf was 3 to 4 liter, age introduce non milk feed and weaning age varied from farms to farms. No special starter feed was used in any of the farms rather straw, hay and concentrate feeds used for calves and 4.2 weeks of age was the average age of introduction of feeds. In most of farms house (83.84%), calf under six months of age was kept in separate group calf pen, but in the rest farm house (16.15%), calves was kept together with the cow in the same barn and in none of the dairy farms bedding materials, separate calving facilities and footbaths were presents.

In most dairy farms regular farm visits were not practiced, however regional and private veterinary practitioners has been visit the farms on call during abnormal health conditions happened in the farm. Among 30 farmers interviewed 21 (70%) of them mentioned that Calf morbidity and mortality is one of health problems, and majority of them complained diarrhea as a major cause of morbidity and mortality.

Morbidity and mortality incidence

The results of this study revealed that the incidence of calf diarrhea and mortality in the first six months of calfhood were 29.39% and 9.69%, respectively (Table 1).

Diseases Condition	No. ofCases	Calf monthat risk	Per calfMonth	Incidence Rate	
				Incidence rate/calf 6 month at risk (I)	Incidence Risk[a] (%)
Diarrhea	27	465.6	0.058	0.348	29.39
Calf mortality	9	514.6	0.017	0.102	9.69

Table 1: The incidence of calf diarrhea and mortality in calves.
a = derived by the formula, risk = $1\text{-}e^{-i}$, (27).

Association of potential risk variables with incidence of diarrhea and mortality

Out of the 13 risk factors analyzed for calf diarrhea, eight factors were found significantly ($P < 0.05$) associated with calf diarrhea in a univariate analysis using logistic regression. These include age of the calves, parity of the dam, age at first colostrum feeding, weaning age, barn ventilation status barn, drainage status manure, disposal system and house cleanness (Table 2).

Variable	Category	Number of calf	Diarrhea (%)	Crude OR (95%CI)	P value	Adjusted OR (95%CI)	P value
Age	>-3month	32	1 (3.13%)		0.006		
	<3month	98	35 (35.71%)	17.22 (2.25-131.62)		0.06 (0.01- 0.72)	0.026
Sex	Female	108	31 (28.70%)		0.569		
	Male	22	5 (22.73%)	0.73 (0.25-2.15)			
birth condition	Normal delivery	101	24 (23.76%)		0.065		
	Dystocia	29	12 (41.38%)	2.26 (0.95-5.40)		0.79 (0.18- 3.37)	0.747
Parity of the dam	First parity	97	20 (20.62%)		0.003		
	2nd parity and above	33	16 (48.48%)	3.62 (1.56-8.41)		3.39 (1.04- 11.01)	0.043
Herd Size	Small size (<10)	39	11 (28.21%)		0.932		
	Large size (>-10)	91	25 (27.47%)	0.96(0.42-0.22)			
Age of the first colostrum ingestion	<_12 hours	99	19 (19.19 %)		0.001		
	> 12 hours	31	17 (54.84%)	5.11 (2.15-12.16)		2.30 (0.68- 7.86)	0.182
Age of weaning	<_3 months of age	109	26 (23.85%)		0.030		
	> 3 months of age	21	10 (47.62%)	2.90 (1.11-7.60)		1.39 (0.30-6.50)	0.676
Housing condition	Separate calf pen	109	27 (24.77%)		0.095		
	Same barn with cow	21	9 (42.86%)	2.28 (0.87-5.99)		1.96 (0.51-7.52)	0.327
Floor type	Soil	98	27 (27.55%)		0.950		
	Concrete	32	9 (28.13%)	1.03 (0.42-2.50)			
Barn ventilation status	Poorly ventilated	52	23 (44.23%)		0.001		
	well ventilated	78	13 (16.67%)	0.25 (0.11-0.57)		0.30 (0.10 -1.900)	0.05
Barn drainage status	Poorly drained	80	12 (15.00%)		0.000		
	Well drained	50	24 (48.00%)	5.23 (2.29-11.96)		2.95 (1.01- 8.65)	0.05
Manure disposal system	Accumulated	107	24 (22.43%)		0.005		
	Regularly disposed	23	12 (52.17%)	3.77 (1.48-9.62)		1.39 (0.39-4.90)	0.613
House cleanness	Clean	100	20 (20.00%)		0.001		
	Unclean	30	16 (53.33%)	4.57 (1.92-10.90)		2.91 (0.90- 9.41)	0.073

Table 2: Potential Risk Variables significantly associated with the incidence of calf diarrhea in a univariable and multi variableanalysis using logistic regression.

Further analysis using multivariant analysis with significant association ($P < 0.25$) with calf diarrhea after univariant analyses indicated that only age of calves, parity of the dam, barn ventilation status barn and drainage status manure were significantly associated with the risk of calf diarrhea. Younger calves and calves from multiple parity dam were at higher risk for diarrhea than older calves (Table 2).

With regard to mortality due to diarrhea, birth condition, parity of the dam, age of the firstcolostrum, manure disposal system and house cleanness were found with significant association ($P < 0.05$) in the univariate logistic regression. Among the five variables significantly associated withcalf mortality in the univariate analysis, only age at first colostrum ingestion were significantly associated with calf mortality ($P < 0.05$) using multiple variant analysis (Table 3). The incidence of mortality was 18.48 times higher for calves, which ingested their first colostrum meal later than 12 hours after birth than those ingested within 12 hours after birth (Table 3).

Bacterial Agents Associated with Calf Diarrhea and antimicrobial susceptibility test Laboratory examination of 36 fecal samples from diarrheic calves that occurred during the study period was done to identify pathogens associated with calf diarrhea. Examination of samples wasdone only for *E. coli* and *Salmonella*. Of the 36 samples examined, 47.22% and 16.67% werepositive for *E. coli* and *Salmonella* respectively (Table 4).

Variable	Category	Number of calf	Mortality(%)	Crude OR (95%CI)	P value	Adjusted OR (95%CI)	P value
Birth condition	Normal delivery	101	24 (23.76%)		0.004		
	Dystocia	29	12 (41.38%)	8.52 (1.98-36.64)			
Parity of the dam	First parity	97	20 (20.62%)		0.043		
	2nd parity and above	33	16 (48.48%)	4.15 (1.04-16.52)			
Age of the first colostrum ingestion	<_12 hours	99	19 (19.19 %)				
	> 12 hours	31	17 (54.84%)	34.10 (4.06-286.23)	0.001	18.48 (1.91-178.31)	0.012
Manure disposal system	Accumulated	107	24 (22.43%)		0.042		
	Regularly disposed	23		4.29 (1.06-17.47)			
			12 (52.17%)				
House cleanness	Clean	100	20 (20.00%)		0.027		
	Unclean	30	16 (53.33%)	4.8 (1.20-19.21)			

Table 3: Potential risk variables that were significantly associated with the incidence of calf mortality based on univariate andmultivariate analyses using logistic regression.

E. coli			*Salmonellae*		Total
	Negative	Positive	Negative	Positive	36
Diarrhea	19 (52.72%)	17 (47.22%)	30 (83.33%)	6 (16.67%)	

Table 4: Bacterial agents Associated with Calf Diarrhea.

E coli were found to be susceptible for chloramphenicol and gentamycin but developed resistance against ampicillin and tetracycline. *Salmonella* was found to be susceptible for chloramphenicoland developed *resistance against three drugs;* gentamycin, erythromycin and amoxicillin (Table 5).

Bacterial isolates			
Drugs	Interpretation*(R-I-S)	*E. coli*	*Salmonellae*
Ampicillin	R	10 (58.8%)	3 (50%)
	I	3 (17.7%)	2 (33.3%)
	S	4 (23.5%)	1 (16.7%)
	Total	17	6
chloramphenicol	R	3 (17.7%)	1 (16.7%)
	I	5 (29.4%)	1 (16.7%)
	S	9 (52.9%)	4 (66.7%)
	Total	17	6
Gentamycin	R	4 (23.5%)	4 (66.7%)
	I	2 (11.8%)	1 (16.7%)
	S	11 (64.7%)	1 (16.7%)
	Total	17	6
Tetracycline	R	9 (52.9%)	3 (50%)
	I	4 (23.5%)	1 (16.7%)

	S	4 (23.5%)	2 (33.3%)
	Total	17	6
Erythromycin	R	7 (41.2%)	4 (66.7%)
	I	4 (23.5%)	0 (0.0%)
	S	6 (35.3%)	2 (33.3%)
	Total	17	6
Amoxicillin	R	8 (47.1%)	4 (66.7%)
	I	6 (35.3%)	1 (16.7%)
	S	3 (17.7%)	1 (16.7%)
	Total	17	6

Table 5: Antimicrobial susceptibility profile of *E. coli* and *Salmonella.*

Discussions

Calf diarrhea and mortality are the major constraints of dairy farming system. In the present study, the cumulative incidence of crude calf diarrhea (29.39%) and calf mortality (9.69%) was estimated in selected dairy farms of study site. Similar observations were also made by other authors [9,10]. In the present study calf mortality was 11.3% which was similar with Bekele., *et al.* [26]. (9.3%) and lower than previously reported by Ferede., *et al.* [[9]. (30.7%). The current study results of calf diarrhea and mortality cumulative incidence report compared to other author showed wider variability due to different study design, different mangemental and environmental condition. Relatively the higher incidence risk of calf diarrhea (29.39%) was recorded, which was in agreement with Wudu., *et al.* [10]. and Bekele., *et al.* [26]. Unhygienic feeding utensils observed during the study might be responsible for the high incidence of calf diarrhea.

Concerning the potential risk factor, a range of explanatory variable was analyzed for their association with calf diarrhea and mortality. Accordingly age of the calves and parity of the dam were found the important factor significantly associated with calf diarrhea. In this study, calves under three months of age were at higher risk of diarrhea which is in agreement with previous studies [10]. On the other hand there are also studies which indicate higher mortality in older calvesthan younger ones [30]. Calves from 2nd and above party dam were at higher risk of diarrhea than calves with single parity dam. Age of the first colostrum ingestion was found significantly associated with calf mortality due to diarrhea. In this study calves which ingested their firstcolostrum meal later than 12 hours after birth were at higher risk of death than those ingested within12 hours after birth. This was in agreement with Wudu., *et al.* [31].

In the present study, *E. coli and Salmonella* were detected from diarrheic calves from dairy farms ofHarar city. Similarly, these pathogens were also detected and isolated from calves by many other Authors [32]. According to the present finding, *E. coli* was the bacterial agent cultured with a frequency 47.22% from diarrheic calves which was comparable to that reported by Khan and Khan [33] (54%) and Dawit, (34) (64%). But, the present result is higher than the report by Herrera-Luna., *et al*. [35] and Haschek., *et al*. [36] who stated isolation rates of 18.9% and 17.9%, respectively. In contrast to this study much higher detection of *E. coli* was reported by Adesiyun., *et al*. [37]. (84.3%) from diarrheic calves in Trinidad. The variation might be due to the difference in the diagnostic technique used.

Salmonella was also the bacterial agent cultured with a frequency of 16.67% from diarrheic calves which was lower than the observations of Caple [38], who found 36% of *salmonella* species from calves. Pergram., *et al*. [4]. also diagnosed *Salmonella* as an important cause of calf diarrhea in dairy farms of Ethiopia. Moreover, the lower detection rate might be due to the fact that shedding of the agent did not coincide with the sampling occasion, failure to detect the causative agent might be due to diagnostic method used and some cases of diarrhea might not be associated with infectious agents but, instead, due to management or nutritional factors. In similar way, the frequencies of *Salmonella* isolation vary from one location to the other due to different managemental and hygienic regimes as well as geographical, environmental and individual differences, Currently antimicrobial resistance is an important issue in public health, animal health and food safety. Thus, antimicrobial susceptibility test was performed to *E. coli* and *Salmonella* isolates. In this study, both *E. coli and Salmonella* isolates were susceptible to chloramphenicol. The current result was different from those of Aksoy., *et al.* [39]. and Ynehiwot [32], who reported high suscep-

tible rate of *E. coli* and *Salmonella* to gentamycin. In the present study E. coli has also been developed some percent of resistance level against gentamycin.

E. coli isolates were relatively resistant to erythromycin. The finding of the erythromycin with resistance was relatively comparable with Nazir, [40] who reported 100% erythromycin resistant *E. coli* isolates. *E. coli and Salmonella* has been developed resistance against ampicillin and amoxicillin which is in agreement with Bradford., *et al.* [[41], and Nasir [40]. The high resistance of these drugs in gram-negative bacteria might be due to the transfer of resistance genes form gram- positive bacteria of β-lactamase genes [42,43].

Salmonella has also been developed resistance against gentamycin, erythromycin and tetracycline The antimicrobial sensitivity patterns of the *Salmonella* isolates in this study was in agreement with Ynehiwot [32] who reported resistance to different antimicrobial agents like erythromycin, tetracycline. The indiscriminate use of different kinds of antibiotics creates a potential health risk toanimals and humans in terms of drug residues and the development of resistant bacterial strains.

Multidrug resistance of *E. coli* and *salmonella* isolates in this study could be largely due to acquired antimicrobial resistance phenotypes most often develop via conjugative transfer of plasmids Di Plasmids may carry class I integrons, which are mobile DNA elements that are important in the proliferation of bacterial multidrug resistance, especially among the gram-negative enteric species [44].

Conclusions

Calf diarrhea and mortality was found to be relatively high in the study area and can have short- term and long-term detrimental effects on dairy production by suppressing growth rate of the calvesand replacement capacity of the herds. It has also been found that both the calf and environmental factor including: age of the calf, age of the first colostrum ingestion and parity of the dam were the most important determinants associated with calf diarrhea and mortality in the study areas. From all antimicrobial agents chloramphenicol was effective to both of *E. coli and Salmonella* isolates. Mostof *Salmonella* isolate were resistance to ampicillin, gentamycin, tetracycline, erythromycin and amoxicillin. Multidrug resistance profile was developed by *Salmonella* and *E. coli* isolates to different antibiotics. Ampicillin, erythromycin, gentamycin, tetracycline and amoxicillin were the most frequent drugs that showed multidrug resistance pattern in all bacterial isolates. Therefore, awareness should be created to dairy producers on good calf rearing and management practicewhich are greatly suggested in reduce the high level of calf diarrhea the study herds and also in other areas with similar management system. The presence of multidrug resistance indicates the need for proper and strict usage of drugs in the future.

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Consent for Publication

I agreed on the publication of this manuscript to Open Access Journal of Veterinary Science & Research and possible to send their consent if needed.

Competing Interests

The authors declare that they have no competing interests.

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