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Original Research Article

Isolation of Common Calves Diarrhoea Causing Bacterial and Protozoal Enteropathogen and Associated Risk Factors from the Dairy Farms in and Around Assela Town Arsi Zone, Oromia Regional State of Ethiopia

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Abstract: Calf morbidity and mortality resulted from diarrhea hinder the replacement of older cows by calves and lower economic benefit from the secretor in Ethiopia. Bacterial and Protozoal infectious disease were the main causes of calves' diarrhea in dairy farms. The aim of the current study was conducted to isolate calf's diarrhea causing Bacterial and Protozoal infection and asses the risk factors associated with it in and around the Assela town. Accordingly, 26 small, medium, and large-scale dairy farms were purposively selected for collection of faecal and serum samples and risk factors related information diarrhoeic calves. From 108 calves, 2 × 10 g of feces and 10 mL of jugular blood from each calf were collected. Bacterial culturing and serodiagnosis and protozoal microscopic examination were conducted. From 26 small, medium, and large dairy farms in the Assela region were investigated for the percentage of significant enteropathogens in diarrhoeal calves over six months. They contained proportions of *E. coli,* Eimeria, Cryptosporidium, Giardia and Salmonella Typhimurium of 55 (50.9%), 42 (38.9%), 23, 8 (7.4%) and 3'(2.8%), respectively. All risk factors assessed had equal impact on the occurrence of Enteropathogen hence there was no significant difference among them (P-value > 0.05). the high calf Diarrhea causing Enteropathogen rates established in this study together with the alarming predictors of calf morbidity entail attention by the concerning bodies on proper management and improved health care to reduce the incidence of Calves diarrhea.

Keywords: Bacterial, Calves, Diarrhoea, Enteropathogen and Protozoal.

1. INTRODUCTION

In Ethiopia, livestock have a substantial importance in economic and social development to the community in sector from the household up to the national level (Tamrat *et al.,* 2020). Dairy Production is one of the most significant livestock sector the Ethiopian governments given the priority to increases the supply of milk and meat from smallholder dairy farms(Tesfaye, 2019)

According to this, initiation for the development of the dairy industry in urban and peri-urban areas was increased in order to fulfill the demand for milks and as on tools to decrease the job opportunity for un employed youth in the country(Hordofa *et al.,* 2021). The sustainability and continuity of livestock production requires the replacement of older cows by calves (Franklin & Jackson, 2002). However, the demand for the calf in Ethiopia is high, calf morbidity and mortality rates resulted from diarrhea and respiratory disease are very high and hinder production, incomes, and the ability of farmers to improve their economic welfares (Wong *et al.,* 2022). Calf mortality is a common problem for livestock producers as it causes loss of future breeding stock and replacement dairy cows, a loss of slaughter cattle, a loss of future draught oxen and a loss of milk production in breeds milked with the calf at foot (Tadesse *et al.,* 2017) The annual direct losses from young ruminant mortality are generally estimated to be 8% to 10% (Mohammed *et al.,* 2020)

Calf refers to the age group of young cattle from birth to nine months of age. Calves are at highest risk for death in the first two weeks of life (Especially in the first week) (Tadesse *et al.,* 2017).. Diarrhea is the most commonly reported calf disease with economic loss due to treatment costs, stunted growth rates and calf mortality (TORCHE *et al*..,2020). Calf diarrhea is a clinical syndrome associated with several infectious and non-infectious diseases that hinder the absorption of fluids from the intestine and causing electrolyte imbalance, rapid dehydration, and acidosis which is fatal for calves (Abebaw, 2018).The two primary diseases are diarrhea and pneumonia and are the commonest disease in young calves andpre-weaned respectively. Even though other diseases like navel ill, arthritis, bloat, arthropod parasites and nutritional diseases feeding problem are also reported (Tadesse *et al.,* 2017). Most of the morbidity and mortality calves in dairy industry is resulted from complicated of the environment, management practices, the animal itself and, infectious agents. particularly colostral feeding, housing, calving assistance, production system, herd size, season, and hygiene of microenvironment were reported as management and environmental factors play significantly role in calf morbidity and mortality(Hadgu *et al.,* 2021).

Morbidity and mortality of young stock present economic and production challenges to livestock producers globally(Wong *et al.,* 2022). In Ethiopia, calf morbidity and mortality were the second biggest problem next to mastitis for dairy production.(Tesfaye, 2019). Despite the huge number of cattle in the country productivity is low due to constraints of disease, nutrition, poor management and poor performance of endogenous breed. This constraint results in poor reproductive performance of dairy cattle and lower economic benefit from the secretor (Tadesse *et al.,* 2017). High rates of disease and death occur in various production systems in Ethiopia, hampering livestock production, reducing incomes, and damaging livelihoods (Wong *et al.,* 2022). A retrospective study undertaken in 2015/16 in major livestock production systems of Ethiopia reported alarmingly high annual losses of young stock from birth-to-weaning age and premature losses in terms of abortion and stillbirth (Hadgu *et al.,* 2021).

Calf mortality and morbidity can be decreased with improving the dairy calf health and management practice that including: proper colostrum management, quality nutrition, good housing, sanitation of the calf 's environment and feeding utensils, and control of potential disease carriers (Alemu *et al.,* 2022). The cross-sectional study conducted by Assen, (2016) in Ethiopia indicated the delivery condition and method of colostrums feeding were playing significant role occurrence of calf mortality and morbidity hence there were no practice of during birth calves treatment practice by farmers. improving the whole herd health system and awareness creation to calve owners to improve the dynamism of their future replacement calve is very important in this area(T. Tesfaye *et al.,* 2020). However, the prevalence and risk factors associated with the calf's diarrhea were high in the study areas during the study period, the information about the causes of calf's diarrhea possessed by stakeholders including: farmers, district Veterinary officer and ministry of Agriculture were very low. Therefore, the objective of this study was targeted to isolate the common bacterial and Protozoal Enteropathogen and assess the associated risk factors in study areas in order to inform the stakeholders and police makers to give attention how to control the calf's diarrhea.

2. MATERIALS AND METHODS

2.1. Study Area

The research work was carried out in selected dairy farms in and around Assela town which is found in the central part of the Oromia National Regional State from November 2013 to April 2014. Assela is the capital of the Arsi zone. The zone lies between 60 45' N to 80 58'N and 380 32' E to 400 50' E. Asella is found 175 km Southeast of Addis Ababa. Arsi zone has a total area of 23,881 Km2 and 22 districts (OFEDB, 2007).Tiyo district is one of the administrative units located in the North-western central part of the Arsi zone. The total area of the district is 665 km2 and it has 21 administrative units of which 18 are Peasant associations and 3 are urban administrative units. Undulating plain, hills, rift valley escarpment, Welkesa valley, and the mountain peak of Chilalo characterize the topography of the district. The altitude of the district ranges from 1500-4105 m. The highest peak in the district is Chilalo Mountain at an elevation of 4005 m above sea level. The district has a tropical highland climate characterized by heavy and erosive rainfall with a long-wet season. The district is divided into four ecological zones, namely high land (31.7%), Mid-altitude (42.5%), temperate highland (20.1%), and low land (5.7%). The annual mean temperature ranges from 15-220c and the mean annual rainfall ranges from 900-1100 mm OFEDB (2007). According to the Arsi zone livestock development and health agency, the current animal population of the district is 70, 967 cattle; 55,237 sheep, 14,157 goats, 8,884 horses, 15,730 donkeys, and 303 camels. Small, medium and large-scale dairy farms are found in this area that supply milk and milk products for human consumption. These dairy farms contain either local or cross breeds depending on the scale of production.

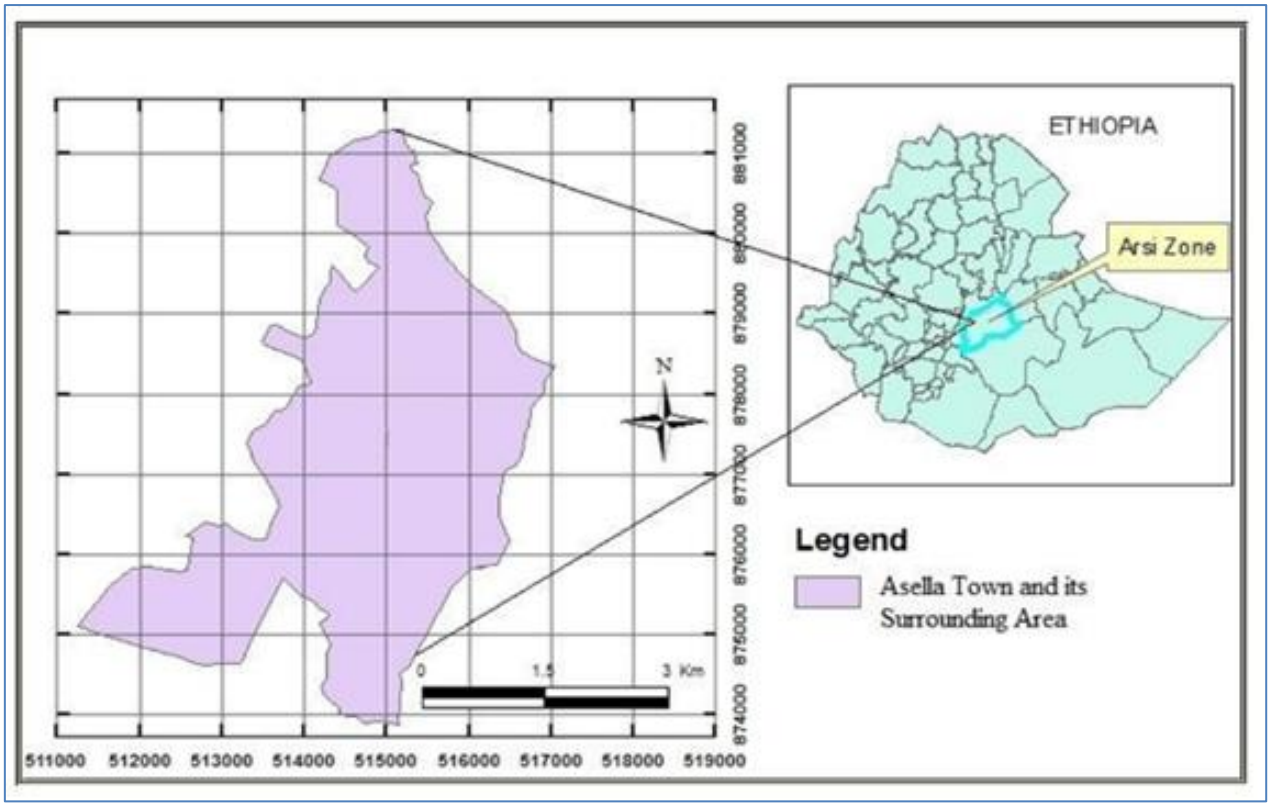

Figure 1: Map showing the location of the study area (Source: Arsi Zone Livestock Development and Health Agency)

2.2. Study Animals Popullation

Animals included in the study consisted of cross-breed calves that were less than four months old and clinically presented with diarrhoea.

2.3. Study Design and Sampling Methodology

A cross-sectional study was conducted that involved collection of diarrheal samples from calves reared in large, medium and small-scale dairy farms of Assela and its surroundings for a period of six months; from November 2013 up to April 2014. A total number of 108 loose feces samples were aseptically collected from purposively selected calves with diarrhea in 26 small, medium, and large-scale dairy farms. approximately 2 × 10 g of feces and 10 mL of jugular blood from each calf was collected from each non-treated diarrheic calves by direct digital stimulation using a disposable latex glove and submitted to Assela Regional Veterinary parasitology and microbiology laboratories in sterile plastic bottles cooled on ice packs for isolation and characterization of *E. coli* and protozoa's (coccidia, cryptosporidium, and *Giardia* spp). Samples were processed on the same day or stored at 4ºC and cultured within 2 days. Isolation and identification of *E. coli* were performed as per procedures described by Merchant and Packer (1967). Blood samples were also collected from diarrheic calves and sera samples were extracted and stored at -20ºC for almost six months. Samples were then transported to Arsho Medical Laboratories Plc for detection of antisera of salmonella.

Isolation, Identification and Biotyping of *E. Coli*

According to the Aklilu *et al.,* (2013). fecal samples were inoculated onto Eosin-Methylene Blue (EMB) agar, incubated overnight at 37°C. Putative *E. coli* was identified by metallic sheen morphology, further identified by Gram staining characteristics, activity on Triple sugar iron (TSI), Methyl-Red (MR), Voges-Proskauer (VP) and Indole biochemical tests. Additionally, fermentation of sugars was tested by inoculating *E. coli* isolates into 1% dulcitol, inositiol, lactose; maltose, raffinose, rhamnose, salicin, sucrose and xylose in peptone water base containing Andradi's indicator. Inoculated tubes were at 37ºC together with controls for seven days and the results were recorded every 24 hours. Production of pink color was considered a positive reaction. Isolates showing similar fermentation reaction patterns on the nine sugars were considered as belonging to samilar biotype.

2.4. Serological Examination of Salmonella

The CROMA TEST-stained antigens are standardized suspensions of killed bacteria prepared for the detection and semi- quantitation by agglutination in slide tests of serum agglutinins. The assay was performed by testing the stained *Salmonella Typhimurium* antigens-somatic blue; flagellar, and red against suspected Serra. After the test reagent

and Sera samples were brought to room temperature, the antigen vial was gently re-suspended and aspirated by dropper several times to obtain a thorough mixture. One drop of the appropriate well-shaken suspension was placed on each circle on a glass test card next to the samples to be tested and the contents were mixed by a disposable stirrer and then rocked gently by hand. Visible agglutination was observed in positive samples and the positive samples were further analyzed by semi- quantitative test using serial dilutions and the titter was reported as the highest dilution that showed agglutination.

2.5. Parasitological Examination of Protozoans

Fecal samples were tested for the presence of *Eimeria* spp, *Giardia* spp and *Cryptosporidium* spp. Laboratory analysis of the fecal samples was conducted using sugar flotation technique. For each sample, 5 ml of feces were processed using sugar solution as the flotation medium to recover *Cryptosporidium* oocysts, *Eimeria* oocysts, and *Giardia* cysts (Ref). In addition, the Modified Ziehl-Neelson Staining technique was conducted for *Cryptosporidium* oocyst (Ref). Microscopic examination was realized using bright-field and phase contrast microscopy. The sample was considered positive for the respective parasite when *Giardia* cysts, *Cryptosporidium* oocysts, and *Eimeria* oocysts were detected in the specimen.

2.6. Data Collection, Management, and Analysis

In current study, a questionnaire on calf rearing practices was involved in dairy calves' diarrhea was performed on the farms with calf's diarrhea cases in and around Asella town. The questionnaire was intended to provide data on risk factors associated with diarrhea of dairy calves in the selected farms. The multiple choice (yes or no) and semi-closed questions was developed. Structured questionnaires and observation of calves' clinical observation was administered to farmers to collect information about the sampled calves including age, sex, herd size, calving facility, colostrum feeding method, colostrum feeding duration, time of first feeding, calf house floor bedding, and floor type in addition to clinical presentation. The collected information was verified and validated at the same time.

Data Analysis

Laboratory results, questionaries' response and clinical case observation was recorded on excel 2010. On excel age of calves were categorized in to four groups 0-7 days, 8-15-day, 16-30 day and represented by 0, 1, 2 and 3 respectively. Also, sex of calves was coded as 0 for Female and 1 for Males. The data were exported to SPSS windows version 20 (SPSS INC. Chicago, IL) for appropriate statistical analysis. The proportion of enteropathogens from the total diarrhoeic calves was determined by using descriptive statistics. Chi-square test was used to measure the association among different variables and the occurrence of diarrhoea SPSS (2010). Effects were reported as statistically significant if P-value was less than 0.05.

3. RESULTS

3.1. Occurrence of Enteropathogens in Calf Diarrhoea

Out of the 108 diarrheic samples examined, three enteropathogen: *E. coli*, *Eimeria* spp, *Cryptosporidium* spp, *Giardia* spp and Salmonella Thyphimurum were isolated in Prevalence of 55(50.9%), 42(38.9%), 23(21.3%), 8(7.4%) and 3(2.8%) respectively with statistically significance differences (P-value < 0.05) (Table 1).

Table 1: Abundance of each enteropathogen in calves dirrhoea

Enteropathogen	(N)	Frequency (%)	X^2	P-value
E. coli	25	26.88	23.6	0.000029
Eimeria	23	24.73		
Cryptosporidium	8	8.6		
Giardia	4	4.30		
Salmonella Thyphimurum	108	2.8		

Eimeria spp is the most dominantly detected enteropathogen 23 (24.73%) next to E. coli 25 (26.88%) as a single ethology whereas Giardia 4 (4.3%) was found to be the least of all examined samples. None of infections were detected in 25 (26.88%) of all examined samples (Table 1).

Table 2: Frequency and proportion of various enteropathogens, and their occurrence as a single form

Enteropathogens	Incidence (%)	X^2	P-value
E. coli	55(50.9)	37.195	0,0000
Eimeria	42(38.9)		
Cryptosporidium	23(21.3)		
Giardia	8(7.4)		

The current study also indicated the concurrent occurrence of calves' diarrhea in study area in which the concurr ent incidence of E. coli was highly abundant when it was compared with the other infection. The occurrence of *E. co*li wi th Eimeria was highly abundant (69.2%) while the occurrence ofEimeria with Crypto was a lower (16.7%) and only *E. co li**Crypto*Eimeria was occurred as concurrent of more than two infection with statistical difference tocauses calves diarr hoea (P-value < 0.05) (Table 3).

Table 3: Proportion of various enteropathogens with *E. coli* and their occurrence as concurrent form Frequency

Pathogen Pattern	No. of observation	Prevalence	X^2	P-value
E. coli with Giardia	8	2 (25%)	47.257	0.0000
E. coli with Eimeria	39	13 (69.2%)		
E. coli with Crypto	26	9 (34.6%)		
Eimeria with Crypto	12	2 (16.7%)		
*E. coli**Crypto*Eimeria	7	2 (28.6%)		

Different risk factors that may contribute to the occurrence of diarrhoea in E. coli infections were analysed and all of the variables were found to be non-significant in this study (P>0.05) (Table 4).

Table 4: Analysis of *E. coli* isolates with different variables

Variables	Level	No of +ve isolate (%)	X^2	P-Value
Calf age	0 (1-7 days)	9(16.4)	3.956	0.266
	1(8-15 days)	9(16.4)		
	2(16-30 days)	12(21.8)		
	3(>30 days)	25(45.5)		
Calf sex	0(Female)	21(38.2)	1.780	0.182
	1(Male)	34(61.8)		
Calving facility	0(Calving pen)	37(67.3)	0.297	0.586
	1(Same barn)	18(32.7)		
Colostrum feeding Method	0(Hand feeding)	41(74.5)	0.111	0.739
	1(Suckling)	14(25.5)		
Colostrum feeding Duration	0(24-48 hr)	12(21.8)	0.809	0.369
	1(>24 hr)	43(78.2)		
Time of first colostrum Feeding	0(<6 hr)	51 (92.7)	0.526	0.468
	1(6-24 hr)	4(7.3)		
Calf house bedding	0(Present)	32(58.2)	0.313	0.576
	1(Absent)	23(41.8)		
Calf house floor type	0(Concrete)	42(76.4)	0.111	0.739
	1(Soil)	13(23.6)		
Herd size	0(<10)	11(20.0)	7.228	0.057
	1(10-20)	7(12.7)		
	2(>20)	37(67.3)		

3.2. Distribution of E. Coli Biotypes in Calf Diarrhoea

All (55) positive E. coli isolates were studied for their fermentation activities on nine sugars and all of them (100%) showed the ability to utilize one or more sugars. Accordingly, the most abundant E. coli biotypes from calf diarrhoea were biotype V whereas the least were biotypes III and XII. Biotypes I and VIII, biotypes II, III, and X, biotype XI, IV, and VI, and biotypes XII and XIII fermented the sugars in the following proportions 4(3.7%), 3(2.8%), 2(1.9%) and 1(0.9%), respectively. 1(0.9%) of the isolates 30 and 63 collected from two calves fermented only a single sugar (xylose and inositol), respectively (Table 4).

Table 5: Pattern of biotype-based isolates on the basis of fermentation reactions of nine sugars

Biotype No.	Total No. of isolates & Proportion	Positive sugars
I	4 (3.7)	Dulcitol and sucrose
II	3 (2.8)	Dulcitol and inositol
III	3 (2.8)	Maltose, sucrose and lactose
IV	6 (5.6)	Dulcitol, raffinose, Rhamnose and Sucrose
V	17 (15.7)	Dulcitol, inositol and Rhamnose
VI	2 (1.9)	Dulicitol, raffinose and xylose
VII	7 (6.5)	Dulcitol, rhamnose and salicin

Biotype No.	Total No. of isolates & Proportion	Positive sugars
VIII	4 (3.7)	Dulcitol, rhamnose and xylose
IX	2 (1.9)	Dulcitol,sucrose and raffinose
X	3 (2.8)	Inositol
XI	2 (1.9)	Rhamnose and lactose
XII	1(0.9)	Xylose
Total	**55 (100)**	

Furthermore, based on the result (Table 5) age has a great association with the occurrence of E. coli biotypes (p-<0.05). Biotype V was most dominantly occurring in 1-7 days of age groups. As age increased the occurrence of E. coli decreased (Table 5).

Table 6: Distribution of E. coli biotypes among different age groups of diarrheic calves X2= 62.77df= 39P-value= 0.008

Biotype	Level (Age groups)				Total
	0 (1-7 days)	1(8-15 days)	2(16-30 days)	3(>30 days)	
I	0(0.0%)	2(11.1%)	2(11.8%)	0(0.0%)	4(3.7%)
II	0(0.0%)	0(0.0%)	0(0.0%)	3(5.3%)	3(2.8%)
III	0(0.0%)	0(0.0%)	1(5.9%)	2(3.5%)	3(2.8%)
IV	0(0.0%)	1(5.6%)	1(5.9%)	4(7.0%)	6(5.6%)
V	9(56.2%)	3(16.7%)	3(17.6%)	2(3.5%)	17(15.7%)
VI	0(0.0%)	0(0.0%)	1(5.9%)	1(1.8%)	2(1.9%)
VII	0(0.0%)	0(0.0%)	1(5.9%)	6(10.5%)	7(6.5%)
VIII	0(0.0%)	1(5.6%)	0(0.0%)	3(5.3%)	4(3.7%)
IX	0(0.0%)	0(0.0%)	0(0.0%)	2(3.5%)	2(1.9%)
X	0(0.0%)	2(11.1%)	1(5.9%)	0(0.0%)	3(2.8%)
XI	0(0.0%)	0(0.0%)	1(5.9%)	1(1.8%)	2(1.9%)
XII	0(0.0%)	0(0.0%)	0(0.0%)	1(1.8%)	1(0.9%)
XIII	0(0.0%)	0(0.0%)	1(5.9%)	0(0.0%)	1(0.9%)
Count within age	16	18	17	57	108
%within age	100.0%	100.0%	100.0%	100.0%	100.0%

X^2= 62.77; df= 39; P-value= 0.00

3.3. Calf Management Systems Practiced and Their Association with the Occurrence of Enteropathogens

Management of the calves in the study farms with variations depending on the conditions of the farms was in general as follows: 29(26.9%) calves were left to suckle their dams and 79(73.1%) of them used hand feeding practice after birth. Thirty (27.8%) of the dairy farms had a herd size of less than 10; the herd size of 19(17.6%) of them was between 10 and 20, and that of 59(54.6%) was greater than 20. The durations of colostrum feeding were 24-48 hrs and >48hrs in 20(18.5%) calves and 88(81.8%) of the calves, respectively. The time of first feeding of colostrum was <6hrs and 6-24 hours after birth in 98(90.7%) and 10(9.3%) of the calves, respectively. Calving pens were used for 70 (64.8%) of the calves; whereas 38 (35.2%) of the calves were born in the same barn as that of their dams. After calving 83(76.9%) calves were housed in a separate pen and 25(23.1%) of them were housed together with their dam in the same barn. Calf bedding was used for 60(55.6%) of the calves, but absent for 48(44.4%) calves. The floor types were concrete and soil in pens of 81(75%) and 27(25%) calves, respectively. All of the farms fed on or allowed to suckle colostrum two times per day (morning and evening).

The associations among different risk factors that may contribute to the occurrence of Eimeria were assessed. Based on the result shown in table 6 there was no association among different risk factors except age. Age has a very strong association with the occurrence of Eimeria (p<0.05) was considered statistically significant. No infection was detected in calves less than 7 days of age in this study (Table 6).

Table 7: Association of Eimeria detection along with different variables

Variables	Level	keys	+ve Isolate (%)	X^2	P-Value
Calf age	0	1-7 days	0 (0)	27.768	0.000
	1	8-15 days	3(7.1)		
	2	16-30 days	4(9.5)		
	3	>30 days	35(83.3)		
Calf sex	0	Female	21(38.2)	1.78	0.182
	1	Male	34(61.8)		

Variables	Level	keys	+ve Isolate (%)	X^2	P-Value
Calving facility	0	Calving pen	27(64.3)	0.008	0.927
	1	Same barn	15(35.7)		
Colostrum feeding method	0	Hand feeding	30(71.4)	0.103	0.748
	1	Suckling	12(28.6)		
Colostrum feeding duration	0	24-48 hr	9(21.4)	0.386	0.535
	1	>24 hr	33(78.6)		
Time of first Colostrum feeding	0	<6 hr	39(92.9)	0.366	0.545
	1	6-24 hr	3(7.1)		
Calf house floor Bedding	0	Present	25(59.5)	0.438	0.508
	1	Absent	17(40.5)		
Calf house floor type	0	Concrete	34(81.0)	1.299	0.254
	1	Soil	8(19.0)		
Herd size	0	<10	11(26.2)	3.569	0.168
	1	10-20	11(26.2)		
	2	>20	20(47.6)		

Similarly, the associations among different risk factors that may contribute to the occurrence of Cryptosporidium were assessed. Based on the result sha own in table 7 age has great association with the occurrence of Cryptosporidium ($p<0.05$). As age increased the occurrence of cryptosporidium also increased. Other risk factors didn't show any significant association (Table 7).

Table 8: Association of Cryptosporidium detection along with different variables

Variable	Level	N	+ve isolate (%)	X^2	P-Value
Calf age	0	16	3(13.0)	12.294	0.006
	1	18	6(26.1)		
	2	17	8(34.8)		
	3	57	6(26.1)		
Calf sex	0	48	8(34.8)	1.105	0.293
	1	60	15(65.5)		
Calving facility	0	70	14(60.9)	0.199	0.655
	1	38	9(39.1)		
Colostrum feeding method	0	79	16(69.6)	0.191	0.662
	1	29	7(30.4)		
Colostrum feeding duration	0	20	2(8.7)	1.869	0.172
	1	88	21(91.3)		
Time of first colostrum feeding	0	98	22(95.7)	0.839	0.360
	1	10	1(4.3)		
Calf house floor bedding	0	60	12(52.2)	0.135	0.713
	1	48	11(47.8)		
Calf house floor type	0	81	17(73.9)	0.018	0.892
	1	27	6(26.1)		
Herd size	0	30	7(30.4)	1.938	0.379
	1	19	6(26.1)		
	2	59	10(43.5)		

The associations among different risk factors that may contribute to the occurrence of Giardia were assessed. Based on the result shown in table 8, age and calf house floor bedding have an association with the occurrence of Giardia ($p\leq0.05$). As age increased the occurrence of being exposed to Giardia also increased. Calves using floor bedding were highly affected than those with no bedding. No calves were affected with less than one month of age and all 8(100%) of calves affected with Giardia were over one month of age (table 8).

Table 9: Association of Giardia detection with different variables

Variable	N	+ve Isolate (%)	X^2	P-Value
Calf age	16	0(0)	7.731	0.050
	18	0(0)		
	17	0(0)		
	57	8(100)		

Variable	N	+ve Isolate (%)	X^2	P-Value
Calf sex	48	3(37.5)	0.169	0.681
	60	5(62.5)		
Calving facility	70	3(37.5)	2.827	0.093
	38	5(62.5)		
Colostrum feeding method	79	6(75.0)	0.015	0.902
	29	2(25.0)		
Colostrum feeding duration	20	5(62.5)	11.076	0.051
	88	3(37.5)		
Calf house floor bedding	60	1(12.5)	6.487	0.011
	48	7(87.5)		
Calf house floor type	81	4(50.0)	2.880	0.090
	27	4(50.0)		
Herd size	30	4(50.0)	3.090	0.213
	19	0(0.0)		
	59	4(50.0)		

Out of 108 sera samples tested against Salmonella Typhimurium only 3/108 (2.8%) of were isolated from three age groups 1-7 days (6.2%), 8-15 days (5.6%) and >30 days old (1.8%) while there was no Salmonella thyphimorum in calves of 16-30 days olds.

Table 10: Indirect neurodiagnostic test results of Salmonella from sera samples of diarrheic calves

Age	No. of Examination	Prevalence	X^2	P-value
0	16	1(6.2%)	7.88	0.048
1	18	1(5.6%)		
2	17	0(0.0%)		
3	57	1(1.8%)		
Total	**108**	**2.8%**		

From three serum samples positive for salmonella thyphimurum, one sample from calves of 1-7 days olds was positive for two types of salmonella thyphimorum antigen O and H while the samples from 8-15 days old was positive for O flagellum antigen and samples from >30 days old was positive for H flagellum antigen of salmonella thyphimurum

Table 11: Prevalence of Salmonella Thyphimurum Flagellum Antigen

Detected Ag	No. of observation	Positive (%)	X^2	P-value
H	73	2 (2.7%)	1.19	0.27
O	34	2 (5.9%)		
Total	**107**			

DISCUSSION

Calf morbidity is an important productivity factor that results in huge economic losses in the success of livestock production in Ethiopia(Mohammed *et al.,* 2020).). Diarrhoea in calves can be caused by a variety of pathogens including bacteria, viruses, protozoa, and intestinal parasites. Rotavirus, coronavirus, enterotoxigenic E. coli, and Cryptosporidium parvum are the four major pathogens associated with neonatal calf diarrhoea worldwide. These organisms are responsible for the vast majority (75%-95%) of enteric infections in neonatal calves worldwide (Tzipori, 2005). In the dairy farms. Among disease conditions/ syndrome that cause of calf mortality in Ethiopia, calf diarrhea was the predominant one which shares 44% of calf loss (Brunauer *et al.,* 2021). Diarrhoea is a leading cause of economic losses to the cattle industry and a major cause of calf mortality and morbidity during the first few weeks of life in most countries (Radostits *et al.,* 2000).so that the aim of this study was to isolate calf's diarrhea causing Bacterial and Protozoal infection and asses the risk factors associated with it in and around the Assela town.

The overall prevalence of *E. coli*, Eimeria, Cryptosporidium, and Giardia in descending order was 55(50.9%), 42(38.9%), 23(21.3%), and 8(7.4%), respectively. The final sum percent of positive calves is over 100% due to concurrent infection (infection with two or more pathogens). *E. coli* as a single cause of diarrhoea is the most dominantly detected enteropathogen (Table1). *E. coli* was detected in more than half of the fecal samples concurrently in the presence of one or more other protozoan enteropathogens as compared to the single cause of the diarrhoea samples in the present study. But since this organism is regarded as a normal member of the intestinal flora of warm-blooded animals, the finding of dissimilarity in concurrent and single infections might be considered indicative of normal flora. Diarrhoea

due to the enterotoxigenic Escherichia coli is one of the most frequent bacterial diseases in neonatal calves and the predominant pathogen cultured from calves with septicemia (Lofstedt *et al.,* 1999). The detection of E. coli with the greatest frequency from diarrhoeic calves in this study is consistent with the results of (Bekele *et al.,* 2009, Bendali *et al.,* 1999, Garcia *et al.,* 2000) but much lower than reports by (Acha *et al.,* 2004, Qais *et al.,* 2011) who have demonstrated the prevalence of E. coli in the feces of young calves in 76% and 64%, respectively in Debrezeit and Addis Ababa and much higher than the results reported by (Dersema, 2008, and Radostits *et al.,* 2000), 13.5%, and 22%, respectively. The reported results variation in Ethiopia as well as in other countries as compared to the present study might be due to variations in the management practices, including hygienic conditions, age groups examined and housing system of the farms, and so on. (Charles *et al.,* 2003) indicated that gaps in management include inadequate nutrition, exposure to the severe environment, insufficient attention to the new born calf, or a combination of these are often involved in scours outbreaks. There was no statistically significant association between E. coli and calves with the practice of hand feeding and the suckling of colostrum.

All positive E. coli isolates were studied for their fermentation activities of nine sugars and all of them (100%) showed the ability to utilize one or more sugars. Among fermented sugars, the most commonly occurring E. coli biotype from calf diarrhoea was biotype V with a prevalence of 17(15.7%) fermented dulcitol, inositol, and rhamnose. This result was consistent with a report by (Gargan *et al.,* 2013) who performed on the same type of sugars to determine biotyping of E. coli and adjacently O-serotyping from rabbit diarrhoea. He assigned as biotype I for an isolate fermenting the above three sugars, which accounted for 66.6% of the isolates, and based on the API 20 E system he concluded that the identified biotype I confirmed with serotyping was ETEC containing O-antigen. So according to him, the most abundant E. coli biotype V of the present study might be ETEC.

Limited information suggests that EPEC strains can cause diarrhoea in calves from 2 days to 4 months of age (Janke *et al.,* 1990). During their first month of life, calves are exposed to many infectious agents for the first time, which alone or in combination with other infectious agents can cause diarrhoea. The pathogen or pathogens encountered in addition to environmental, nutritional and management practices, all influences the duration, severity, and outcome of the disease (Luzoan *et al.,* 1999). Analysis of the relationship among different E. coli biotypes with different age groups of the calves were statistically significant. The biotype test result showed age group 1-7 days was affected at a high rate (9, 56.2%), followed by 8-15 days old (3, 16.7%), 16-30 days old (3, 17.6%) and >30days old (2, 3.5%). This result is consistent with (Lofstedt *et al.,* 1999) who stated that young neonates under one week of age are particularly susceptible because the normal flora of the intestine is not fully established. In addition, they have a naïve immune receptor and also possess for the adhesions of E. coli (Villarroel, 2009).

(Levine *et al.,* 1987) stated that a given strain from diarrheic rabbit (that fermented dulcitol, rhamnose and xylose) and assigned as biotype VI isolated at the rate of 17.6%, was able to induce watery diarrhoea and high mortality after experimental infection. This was in consistent with biotype VIII at the rate of 4(3.7%) of the present study. Similar observations have been made with other strains in England (Varga *et al.,* 1982), Belgium and the (France Camguilhem *et al.,* 1986 and Netherlands Peeters *et al.,* 1984). None of these strains produced heat-labile or heat-stable enterotoxins, nor were they enteroinvasive. So, they are considered to be enteropathogenic E. coli, according to the definition of (Levine *et al.,* 1987). Histology and electron microscopy showed these strains to be tightly adherent to the brush border of intestinal epithelial cells after experimental infection and to cause effacement of microvilli, followed by epithelial desquamation, villous atrophy, and malabsorption (Levine *et al.,* 1987). So, it could be considered that biotype VIII of the present study might be EPEC.

The total infection as a single Forms 60(55.5%) and none infections were detected in 25(26.88%) of all examined samples (Table 1). Concurrent infections of protozoan enteropathogens with E. coli in different age groups of calves and sex were also assessed and the result revealed no significant association in all of the episodes (P-value >0.05) (Table 2). Bovine coccidiosis is an important protozoan disease of genus Eimeria affecting calves all over the world resulting in considerable economic losses each year to the beef and dairy industries (Daugschies and Najdrowski, 2005). The prevalence of Eimeria was 38.9%, which is lower than previous findings reported in Addis Ababa and Debre Zeit by (Abebe *et al.,* 2008) (68.1%) and in South Africa by (Matjila and Penzhorn, 2002) (70%) and higher than Kebadu (1998) (20%) in Debre Zeit, and Bekele *et al.,* (2012) (22.7%) in Dire Dawa, Pfukenyi *et al.,* (2012) (19.8 %) from Zimbabwe and Gillhuber *et al.,* (2014) (13.3%), from Southern Germany. This variation is most likely attributed to the differences in agroecology, and husbandry practices of the study animals in different countries Radostits *et al.,* (2007). There was a strongly significant association (P<0.05) between the age of the calves with the risk of infection in Eimeriosis. This finding agrees with the report by Alemayehu *et al.,* (2013) and Bekele *et al.,* (2012).

Cryptosporidium has been identified in calves worldwide, with infection rates up to 100%. Infection can occur in calves as young as 4 days of age. However, the parasite is most commonly identified in calves between 8 and 21 days of age Fayer *et al.,* (1997). Cryptosporidium oocyst was detected in 23(21.3%) diarrheic calves in this study. This result

is comparable to the findings reported in previous studies from other countries: Naciri *et al.*, (1999); 25.6% by Kvac *et al.,* (2006); Al-alousi and Mahmood (2012) 17.6%, in Ethiopia by Abebe *et al.,* (2008); 27.8% by Alemayehu *et al.,* (2013). A lower prevalence of Cryptosporidium oocyst has been reported in western Canada (Gow and Waldner 2006). The association between the occurrence of diarrhoea and Cryptosporidium infection analysis revealed a (P<0.006), indicating a strong association between the infection with Cryptosporidium and the occurrence of diarrhoea in calves. This is consistent with the results of Gillhuber *et al.,* (2014) in Southern Germany. Cryptosporidium was the only pathogen identified concurrently with Eimeria and E. coli in the present study. The result from this study suggests that Cryptosporidium is one of the major etiological agents of neonatal diarrhoea in calves, this might be due to several factors like early contamination soon after birth by contact with their dams, contaminated litters, asymptomatic carriers, and contaminated environment Castro-Hermida *et al.,* (2002).

Another protozoan parasite, Giardia, has recently emerged as a potentially important parasite of cattle. Many studies have identified Giardia in domestic livestock with a prevalence of up to 89% reported in calves Xiao (1994). Giardia infection in cattle is often subclinical or asymptomatic, but this infection can also cause symptoms including acute or chronic diarrhoea, reduced weight gain, and ill thrift in young calves Geurden *et al.,* (2010). Giardia cysts were detected in 4(7.4%) in the present study. It is significant, that age had a nonlinear influence on the probability of being infected with Giardia speciies. The presence of calf house floor bedding has a significant association (P<0.011) with giardia occurrence; this may be attributed to the explanation that removal of bedding would also be expected to decrease fecal material harboring Giardia Waltner-Toews *et al.,* (1986); and previous studies have concluded that cleaning is an important management factor in preventing high levels of Giardia-cysts Mohammed *et al.,* (1999). The sex and other management practices of the calves were analyzed and not significantly associated (P>0.05) with the infection by Giardia (Table 8). In addition, calves were classified in age groups and sex to realize the association between the occurrence of diarrhoea with four enteropathogens (E. coli, Eimeria, Crypto, and Giardia) but no statistically significant association was observed in all of the events (P>0.05) (Table 2).

Salmonella infections in calves continue to be a major problem worldwide. Substantial economic losses were manifested through mortality and poor growth of infected animals as well as the hazard of transmitting food poisoning to humans. Many outbreaks of salmonella infections have been reported worldwide, the most frequently isolated serovars being S. Typhimurium, S. enteritidis, S. anatum S. Newport, S. Cerro, S. Montevideo, S. agony, and S. dublin which was considered the major host-adapted salmonella for cattle Mitz *et al.,* (1981); Konrad *et al.,* (1994). Salmonella Typhimurium is the most common species. To infect calves. Generally, calves over two weeks of age are most likely to become infected by salmonella Radke *et al.,* (2002). Out of 108 sera samples collected from diarrheic calves, 3(2.8%) of the analyzed samples in the present study were positive against S. Typhimurium both O (somatic) and H (flagellar) antigens, and the titration results revealed recent infection in all of the three positive calves. This result was lower as compared to the result reported for S. Typhimurium by Siorvanes *et al.,* (2007).); (7.3%). The variation of the results in the present study might be attributed to the diagnostic techniques, management, and environmental conditions of the calves because the infection was always aggravated by poor hygienic conditions and inadequate nutrition and young calves are most susceptible to infection due to their immature immune responses, undeveloped microflora in their gastrointestinal echo-system and the permanent exposure to the source of infection from the environment and their dams Radke *et al.,* (2002).

4. CONCLUSION AND RECOMMENDATIONS

The current study identifies common protozoal and bacterial enteropathogen with its prevalence and its risk factors in study area. The prevalence of *E. coli* in calf's diarrhoea was higher than the another enteropathogens isolated in current study. However, there was no significant difference among the assessed risk factors except the age factor, the prevalence of diarrhoeal causing Enteropathogen was shown considerably variable among different enteropathogens. The study also indicated that the prevalence diarrhoea in calves >30 day old was higher another age groups.

Based on the above conclusion, the following recommendations are forwarded:

- ❖ Strict calf's management practice should be followed by all farmers.
- ❖ Farmers should be trained on the causes and prevention of calves' diarrhoea
- ❖ The strains of in-calf diarrhoea should be confirmed using modern diagnostic techniques
- ❖ Further studies are necessary to identify viral enteropathogens in the study area for a complete understanding of pathogens of calf diarrhoea.
- ❖ Further molecular studies should be conducted to identify protozoal enteropathogens at a molecular Level.

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