Sero-Epidemiological Study on Bovine Rota and Coronavirus in Neonatal Calves in Dairy Farms of Addis Ababa, Ethiopia

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Research article

Keywords: Calf diarrhea, Rotavirus, Coronavirus, Risk factors, Addis Ababa

Posted Date: March 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17360/v1

Abstract

Background: Bovine rotavirus (BRV) and coronavirus (BCoV) are most commonly associated etiologies from viral causative agents of neonatal calf diarrhea. A cross-sectional study was carried out in randomly selected dairy farms of Addis Ababa from November 2018 to April 2019 with the objectives of estimating the prevalence of bovine rota and coronavirus infection in diarrheic and non-diarrheic calves and determining the associated risk factors. A probability proportional to size sampling technique (PPS) was used and accordingly, the study involved a total of 110 calves under 30 days of age from 57 dairy herds of which 34 (59.6%), 18 (31.6%) and 5 (8.8%) were small scale, medium scale and large scale farms respectively. As well risk factors associated with herd and calves were obtained from an interview of farm owners.

Results: By using sandwich enzyme-linked immunosorbent assay (ELISA) test, out of these 110 calves, 4 (3.64%) were found positive for rotavirus while only 1 (0.91%) was found positive for coronavirus infection. Data generated from both interview and laboratory investigation was analyzed using SPSS version 20. As a result, prevalence of rotavirus infection showed a significant difference ($P<0.05$) with respect to sex, diarrhea status, colostrum timing and consistency of diarrhea. However, prevalence of coronavirus infection showed significant difference ($P<0.05$) only with consistency of diarrhea.

Conclusions: In conclusion, rotavirus and coronavirus infection in neonatal calves in this finding showed as there is less prevalence in dairy farms of the study area; therefore, effort should be initiated for further studies on other (infectious and non-infectious) causes of calf diarrhea in this area.

Introduction

Ethiopia basically constitutes an agrarian society of which a socio-economic activity of about 85% of the populations is based on farming and animal husbandry. Livestock plays an important role for the majority of the Ethiopian population [41]. Dairy production is one of the critical issues in Ethiopian livestock -based society where livestock and its products are important sources of food and income. Because of better availability of milk market, most of the dairy farms are concentrated in urban and pri-urban areas of the country. The substantial demand-supply variance in milk and milk products for the major urban centers in Ethiopia is a great opportunity for the development and flourishing of peri-urban dairy farms [1]; [16].

The success of any breeding program as well as the future of dairy farms depends upon the rate of survival of calf produced and accordingly calf morbidity and mortality are of great concern of dairy man because most of the dairy farms are confronted with acute problems of calf morbidity and mortality [39]. Neonatal calf diarrhea is one cause of calf mortality and morbidity. It is a complex of different disease syndromes that causes economic losses directly through mortality and cost of treatment and indirectly from poor growth [29]

Calf diarrhea is caused as a result of several environmental, management-related, nutritional and physiological factors either alone or in synergy with different infectious agents such as protozoans, bacteria, and/or viruses [4]. Among these infectious agents that have been implicated in calf diarrhea, viruses like bovine coronavirus (BCoV), bovine rotavirus (BRV), and bovine viral diarrhea virus (BVDV), bacteria's like *Salmonella* species, *E. coli* $K99^+$ and *Clostridium* species and protozoa like *Cryptosporidium* species are some examples [7]; [8]; [33]. Among the viral agents, bovine rotavirus (BRV) and bovine coronavirus (BCoV) are the most commonly associated causes of neonatal diarrhea [27].

According to [19] calf morbidity and mortality was ranked next to mastitis as the second biggest problem for dairy production in Ethiopia. A study done on agents associated with neonatal diarrhea in Ethiopia dairy farms revealed a detection of BCoV, BRV and *E. coli* $K99^+$, 38.9%, 16.7% and 11.1% respectively with no evidence of cryptosporidial infection [2]. Another study on enteropathogens associated with calf diarrhea in dairy farms of Muke-turi, Debre -tsigie and Fitche towns of North Shewa Zone reported prevalence of *E. coli,* salmonella and cryptosporidium as 69.5%, 25.7% and 27.6% respectively [40].

Though, some studies like the above reports done on general causative agents of calf diarrhea in Ethiopia and in some parts of it, there is still a research gap on major viral causative agents of neonatal calf diarrhea in the urban parts of Ethiopia especially in Addis Ababa. Therefore, the objectives of this study were:

- To estimate the prevalence of *bovine rota* and *coronavirus* infection in diarrheic and non-diarrheic calves
- To determine the associated risk factors for BRV and BCoV in dairy farms of Addis Ababa

Material And Methods

Study Area

The study was conducted in Addis Ababa which has 117 woredas, 10 sub-cites in which eight of them (Bole, Kolfe Keranio, Yeka, Gullele, Nifas silk-Lafto, Arada, Addis Ketema and Akaky Kality) were visited during the study period. The city is located at a latitude and longitude of 9°1'48"N and 38°44'24"E respectively [24] and at an altitude of 2500m above sea level. It receives an average annual rainfall of 1800 ml and temperature of 21°c. The relative humidity varies from 70 to 80% during the rainy season and from 40 to 50% during the dry season [9]. The economic activities in Addis Ababa are diverse; according to official statistics from the federal government 16,602 peoples are engaged in agriculture. In addition to the residents of rural parts of Addis Ababa, the city dwellers also participate in animal husbandry and cultivation of gardens [24]. Addis Ababa and its peri-urban areas have 62,166 bovine, 22,647 ovine, 7,531 equine, 5,597 caprine and 330,000 avian species [9].

Study animals

The study animals were neonatal calves in dairy farms up to 4 weeks of age that have or haven't experienced diarrhea which were pure Holstein Frisian or cross breed kept under semi-intensive or intensive management system. All calves with and without history of diarrhea which was born before the study and during the study period less or equal to 30 days old were sampled.

Study design, Sample collection and examination

The study design was cross-sectional type. One hundred ten (110) fecal samples of approximately 32 grams were collected in sterile tube after cleaning of the anal area with a paper towel from both diarrheic and non-diarrheic calves using disposable latex glove. Samples were placed into sterile universal bottle and labeled; transported to a laboratory by ice box containing ice packs for laboratory analysis. The collected samples were stored at +4°C until time of processing. At the time of sampling the name of the farm, animal ID, sex, age, breed, presence of diarrhea, mortality, consistency of diarrhea, colostrum feeding and timing including its amount and management types were recorded for each calf on proper recording format. Diarrhea was considered if faeces are semi-liquid to liquid, with or without other abnormal characteristics such as presence of blood. Any calf with faeces without these characteristics was considered non-diarrheic or healthy.

Fecal samples allowed to thaw at room temperature and diluted volume by volume into dilution buffer until it allows the pipetting of fecal suspensions. Any gruds were discarded by natural decantation for about 10 minutes. Multiscreen Ag ELISA Calf digestive (BIO K 314/1, Belgium) was used to detect BCoV and BRV antigens in the fecal suspensions. The sandwich ELISA procedure was performed according to the manufacturer instruction as detailed in the kit (Kit reference BIO K 314/1). Any sample that yields a difference in optical density (OD) that is greater than or equal to 600% (6) for rota and 700% (7) for coronavirus was considered positive. The test is validated only if the positive control antigens for corona and rotavirus yield difference in the OD at 10 minutes that was >1000.

Data analysis

Data that was generated from laboratory investigation and interview of farm owners was entered and coded into Microsoft Excel 2007 spread sheet and analyzed using SPSS version 20. χ^2 value was calculated using descriptive statistics model. The result was considered to have significant difference if $P < 0.05$.

Results

Overall Prevalence

The overall prevalence of bovine rota and coronavirus accounted was 4.5%. As illustrated in Table 1 below, 3.64% and 0.91% prevalence for rota and coronavirus was found respectively.

Table 1: Overall prevalence of rota and corona virus in neonatal calves

Viruses	№ sampled	№ positive	%Prevalence [95%CI]
Rota virus	110	4	3.64[3.6,3.7]
Corona virus	110	1	0.91[0.90,0.92]
Total	110	5	4.55[4.43,4.56]

Herd and Individual Level Prevalence for Bovine Rotavirus

The prevalence of rotavirus was high in male (7.3%) and in 11-20 days of age (7.9 %) calves. The prevalence was also significantly different (P<0.05) with diarrhea, consistency of the diarrhea, colostrum timing and sex. However, the difference was statistically insignificant (P > 0.05) in the remaining individual and herd risk factors (Table 2 and 3).

Table 2: Association of herd risk factors with rotavirus prevalence

Risk Factors	№ of herd examined	№ of positive herd (%)	% Prevalence [95%CI]	χ2	P -Value
Herd size				2.14	0.343
Small scale	34	3	8.8[8.65,8.95]		
Medium scale	18	0	0.0[0.0,0.0]		
Large scale	5	0	0.0[0.0,0.0]		
Total	57	3	5.3[5.21,5.4]		
Diarrhea				6.86	0.009
Yes	18	3	16.7[16.43,16.96]		
No	39	0	0.0[0.0,0.0]		
Total	57	3	5.3[5.21,5.4]		
Mortality				3.28	0.07
Yes	28	3	10.7[10.52,10.88]		
No	29	0	0.0[0.0,0.0]		
Total	57	3	5.3[5.21,5.4]		
Colostrum feeding				0.98	0.323
Bucket	49	2	4.1[4.01,4.19]		
Suckling	8	1	12.5[12.14,12.8]		
Total	57	3	5.3[5.21,5.4]		
Colostrum timing				6.8	0.033
< 1hr	29	0	0.0[0.0,0.0]		
1-2hr	19	1	5.3[5.14,5.46]		
> 2hr	9	2	22.2[21.8,22.62]		
Total	57	3	5.3[5.21,5.4]		
Bedding				1.35	0.246
Yes	40	3	7.5[7.37, 7.63]		
No	17	0	0.0[0.0,0.0]		
Total	57	3	5.3[5.21,5.4]		
Colostrum amount				0.89	0.64
< 1L	24	2	8.3[8.13,8.47]		
1-2L	28	1	3.6[3.5,3.7]		
>2L	5	0	0.0[0.0,0.0]		
Total	57	3	5.3[5.21,5.4]		
Calf pen				0.14	0.71
Individual	25	1	4[3.88,4.12]		
Group	32	2	6.2[6.07,6.32]		
Total	57	3	5.3[5.21,5.4]		

Table 3: Association of individual risk factors with rotavirus prevalence

Risk Factors	№ of calf sampled	№ of positive calf	%Prevalence [95%CI]	χ2	P -Value
Sex				4.15	0.04
Male	55	4	7.3[7.19, 7.41]		
Female	55	0	0.0[0.0,0.0]		
Total	110	4	9.5[9.42,9.58]		
Diarrhea				6.72	0.01
diarrheic	42	4	9.5[9.36,9.63]		
non-diarrheic	68	0	0.0[0.0,0.0]		
Total	110	4	9.5[9.42,9.58]		
Age				0.47	0.79
1-10d	31	1	3.2[3.1,3.3]		
11-20d	39	3	7.9[7.77,7.99]		
21-30d	40	0	0.0[0.0,0.0]		
Total	110	4	9.5[9.42,9.58]		
Consistency of diarrhea				30.97	0.00
Normal					
Smooth and mucoid	87	0	0.0[0.0,0.0]		
Watery	5	0	0.0[0.0,0.0]		
Bloody	13	4	30.8[30.41,31.19]		
Total	5	0	0.0[0.0,0.0]		
	110	4	9.5[9.42,9.58]		

Herd and Individual Level Prevalence for Bovine Coronavirus

Eventhough bovine coronavirus was detected in: Small scale farms, herd with diarrhea, farms with bucket type colostrum feeding, farms that use to feed colostrum 1-2 hrs after birth and farms feeding <1 liter per day, the herd prevalence was having insignificant difference among those risk factors (Table 4). Moreover, the individual level prevalence was also not significantly different among all risk factors except for consistency of diarrhea (Table 5).

Table 4: Association of herd risk factors with coronavirus prevalence

Risk Factors	№ examined	№ positive	% Prevalence [95%CI]	χ2	P -Value
Herd size				0.69	0.59
Small scale	34	1	2.9[2.81,2.99]		
Medium scale	18	0	0[0.00,0.00]		
Large scale	5	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Diarrhea				2.2	0.14
Yes	18	1	5.6[5.44,5.76]		
No	39	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Mortality				1.05	0.31
Yes	28	1	3.6[3.49,3.71]		
No	29	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Colostrum feeding				0.17	0.68
Bucket	49	1	2[1.94,2.06]		
Suckling	8	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Colostrum timing				2.07	0.36
< 1hr	29	0	0[0.00,0.00]		
1-2hr	19	1	5.3[5.14,5.46]		
> 2hr	9	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Bedding				0.43	0.51
Yes	40	1	2.5[2.42,2.58]		
No	17	0	0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Colostrum Amount				1.4	0.5
< 1L	24	1	4.2(4.08,4.32]		
1-2L	28	0	0.0[0.00,0.00]		
>2L	5	0	0.0[0.00,0.00]		
Total	57	1	1.8[1.75,1.85]		
Calf pen				0.79	0.37
Individual	25	0	0.0[0.00,0.00]		
Group	32	1	3.1[3.01,3.19]		
Total	57	1	1.8[1.75,1.85]		

Table 5: Association of individual risk factors with coronavirus prevalence

Risk Factors	№ sampled	№ positive	% Prevalence [95%CI)	χ2	P -Value
Sex				1.01	0.32
Male	55	0	0.0[0.00,0.00]		
Female	55	1	1.8[1.75,1.85]		
Total	110	1	0.91[0.88,0.94]		
Diarrhea				1.6	0.2
diarrheic	42	1	2.4[2.39,2.47]		
non-diarrheic	68	0	0[0.00,0.00]		
Total	110	1	0.91[0.88,0.94]		
Age				1.9	0.38
1-10d	31	0	0.0[0.00,0.00]		
11-20d	39	1	2.6[2.52,2.68]		
21-30d	40	0	0.0[0.00,0.00]		
Total	110	1	0.91[0.88,0.94]		
Consistency of diarrhea				17.49	0.01
Normal					
Smooth and mucoid	87	0	0.0[0.00,0.00]		
Watery	5	1	20[19.5,20.54]		
Bloody	13	0	0.0[0.00,0.00]		
Total	5	0	0.0[0.00,0.00]		
	110	1	0.91[0.88,0.94]		

Discussion

The result of the present study showed that BRV was highly prevalent, 3.64% (4/110) than BCoV in the study area. There are different rate of BRV infection report in many parts of the world at different times. Eventhough lesser, this result is nearly in agreement with [28] in Costa who reported a prevalence of 7%. Nevertheless, the rate was inconsistent with 25.1% by [21] in Brazil, 20.2% by [4] in Brazil, 22.8% by [42] in Tunisia, 21.84% by [3] (Akam *et al.,* 2011) in Algeria, 15.68% by [30] in India and 15.5% by [5] (Al-Robaiee and Al-Farwachi, 2013) in Iraq. Also much higher rate of 34%, 42%, 42.7% and 50% was reported by [25]; [31] (Reynolds *et al.,* 1986) in England, [14] in Spain and [34] in Scotland respectively. In Ethiopia one study done by [2] revealed 16.7% which was higher than the current finding. This variation may be due to the lower sample size of this study and difference in the test techniques employed.

On the other hand, BCoV was detected at a rate of 0.9% (in only 1/110 samples). This result is lesser when compared to those reported by [34] [13] in India, [14]; [28]; [30] and [31] that was 4%, 4.76%, 7.34%, 9%, 11.76% and 14% respectively. Much higher rate 19% and 21.6% was also reported by [36] in Brazil and [20] in Australia respectively. But almost in agreement with 1.96% (1/51) reported by [17] in northern Turkey. The variation in prevalence rate among reports may be due to difference in farm management practice, hygienic condition and diagnostic techniques employed [23]; [26].

The association of BRV and BCoV infection was found different in different herd and individual level risk factors. Regarding sex, equal number of male (55) and females (55) were sampled in the study and the

occurrence of the infections in male (7.3%) is higher than that of in female (1.8%). [11] and [10] also reported that as compared to females, male susceptibility for diarrhea was high. This can be explained as size of male at birth is assumed to induce dystocia and consequently decrease colostrum absorption. Plus more care is given to female calves than males because of their economic importance. In contrast, the result reported by [6] and [18] showed that the percentage of females affected by rotavirus is higher than male calves.

The prevalence of both BRV and BCoV was high in calves at the age of 11-20days, 3 (7.9%) and 1(2.6%) respectively. This may be due to lack of natural immunity against the two infections and a decrease in passive immunity [35] ;[38] and [6]. In calves' 1-10days of age, only infection by rotavirus was detected 1(3.2%). This may be because there was a good neonatal calf care given at this age range in the study area. None of the pathogens were detected in the third age group, 21-30days. These may be justified as: increased natural immunity against the pathogens as calves reach and become beyond 3 weeks old [17]; [37].

All the positive samples for both pathogens were detected from the diarrheic calves (9.5% for BRV and 2.4% for BCoV). The prevalence was significantly different between diarrheic and non-diarrheic calves and its consistency. None of the non-diarrheic calves showed infection by both pathogens in the present study. A study done elsewhere by [12] and [17] revealed no rota and coronavirus antigen detection in non-diarrheic calves respectively. Moreover, the consistency of diarrhea for the positive samples was watery for all rota positives and smooth for the corona positive which is due to effect of the viruses mainly on the small intestine villi [32];[22].

The time for clostrum feeding, feeding types and its amount were also considered in this study. The prevalence of BCoV (2%) and BRV (4.1%) was high in herds that feed colostrum with bucket than suckling. This may be due to contamination of buckets with calf feces that also contaminate colostrum while feeding calves. Calves getting colostrum above 2hrs had shown a high prevalence rate of 22.2% for rotavirus while 5.3% prevalence rate for coronavirus was detected in calf feeding within 1-2hrs in the herd. This may be due to: decline in immunoglobulin transfer across the gut epithelium as the timing increases as well as less frequent adequate colostrum feeding by the farms within the first 12 hours postpartum (even after the first colostrum feeding). A high infection rate of 8.3% and 4.2% for BRV and BCoV was found in farms that give colostrum below 1litter respectively. This can be justified as low amount of colostrum provision to the calves. Rate of FPT decreases in calves that receive >100gram of colostral IgG and therefore adequate amount of this IgG mass (>100gram IgG) can be achieved if the calf received 4liter of colostrum or minimum of 3liter [15].

In conclusion, data from this study showed that both BRV as well as BCoV infection were involved in neonatal calf diarrhea. Therefore, it is advisable if: awareness would be created to dairy farm owners and attendants on overall farm management specifically on proper cow-calf management practice in dairy farms, further studies on other (infectious and non-infectious) causes of calf diarrhea would be carried out and proper vaccination program could be designed for protection of calf diarrhea.

Declarations

Ethics approval and consent to participate; the research was ethically reviewed and the use of animals was approved by the Ethics committee of the Jimma University College of Agriculture and Veterinary Medicine. Consent of the owners was obtained to use their animals.

Consent for publication; Not applicable" in this section.

Competing interests; the authors declare that they have no conflict of interest.

Funding; this study was not financially funded but supported by vehicle for data collection and also supported in case of chemicals and reagents used in laboratory processing of data by National Animal Health and Diagnostic Center and Jimma University, College of Veterinary Medicine and Agriculture.

Availability of data and materials; the datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgement and Authors' contributions; I want to thanks institute of National Animal Health and Diagnostic Center and Jimma University, College of Veterinary Medicine and Agriculture. Also I Thank my main advisor Dr. Motuma Debelo and co advisor Dr. Asaminew Tesfaye for their continuous support, sincere guidance, idea oriented discussion as well as correcting of this paper.

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