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Research Article

Isolation and Molecular Detection of Rota Virus Associated with Calves, in Central Oromia, Ethiopia

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Abstract

Epidemiological study, conducting of the diagnostic tests, clinical observation and molecular Detection of the causative agent is important for prevention and control of the diseases. Diarrheal disease in calves caused rotavirus which the most common pathogen. Despite the fact that, the effect of rotavirus which was cause calves diarrhea in Ethiopia are not fully understood. A cross-sectional study was undertaking with the purpose of isolation and molecular detection of rotavirus in calves from central part of Oromia (Bishoftu, Sebata, Holeta and Addis Ababa), Ethiopia from November 2018 to May 2019. The study sites were selected purposively and fecal samples were collected for detection of rotavirus by using antigen detection Enzyme linked immunosorbent assay (Ag-ELISA) kit. The associated risk factors of rotavirus infection were also studied. Different farm levels (floor of the calf) and calf level (sex, age) factors were measured the level of association between variables. In the current study, 245 (83 diarrheic and 162 non-diarrheic) fecal samples were collected from calves less than 1 month of age and then samples were screened for presence of rotavirus infection. From 83 diarrheic samples, 6 (7.2%) samples were found positive for rotavirus antigen by Ag-ELISA. But all 162 non-diarrheic samples were negative for rotavirus infection. 2.45% (6/245) were an overall prevalence of rotavirus in calves. All samples (6) of ELISA test positive were propagated in Madin Darby Bovine Kidney Cell (MDBK cells). After 3 continuous passages, progressive cytopathic effect (CPE) of 4 sample (66.7%) were founded. For detection of rotavirus by molecular technique, reverse transcriptase polymerase chain reaction was used to show the occurrence of rotavirus by using specific generic primers for VP4 genetic regions in feces samples. From 6 sample which was positive by ELISA, only Four samples, were develop CPE on cell culture and positive on RT-PCR technique. In male calves the peaked of infection prevalence was obtained at first and second weeks of age. Rotavirus which was lead diarrheal disease in calves have zoonotic importance, decreased weight of calves and increased mortality. So, the current study showed rotavirus disease in calves in Ethiopia that should be solved by practicing early colostrum feeding in newborn calves and improving husbandry system of livestock.

Keywords: Calf Diarrhea; Cytopathic Effects; ELISA Test; Rotavirus; RT-PCR Reactions

Abbreviations

Ag: Antigen; Ag-ELISA: Antigen Capturing Enzyme-Linked Immunosorbent Assay; BRV: Bovine Rotavirus; cDNA: Complementary DNA; CPE: Cytopathic Effect; DNA: Deoxy Nucleotide Ribonucleic Acid; ELISA: Enzyme-Linked Immunosorbent Assay; FAO: Food and Agriculture Organization; MDBK: Madin Darby Bovine Kidney

Cell; mRNA: Messenger RNA; NAHDIC: National Animal Health Diagnostic and Investigation Center; NCD: Neonatal Calf Diarrhea; NSP: Non-Structural Proteins; NVD: No Virus Detected; OD: Optical Density; p.i: Post Infection; PBS: Phosphate Buffered Saline; PCR: Polymerase Chain Reaction; RNA: Ribonucleic Acid; rpm: Revolution Per Minute; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; RVA: Rotavirus Group A

Introduction

Rearing of healthy young stock represents an important indicator of future animal production for worldwide since replacement of cows for the future dairy and beef herd sustainability. Even though dairy and beef industry are a crucial component of agro-based economy for a country like Ethiopia, new born animals suffer with fairly higher mortality and it is one of major reprisal over economy in livestock industry. Among the factors that was protecting the success of dairy industry is morbidity and mortality of calves which leads economic losses on dairy farms worldwide. Also, according to International Livestock Center for Africa (1994) now a day called ILRI, calf morbidity and mortality were as the second biggest problem for dairy industry in Ethiopia. Intestinal problems are the major areas of health problems in early phase of calves, and responsible for weight loss, reduced growth and a higher first calving age, all cost the farmers economically [3].

Calves' diarrhea is one of the most challenging clinical syndromes throughout the world. Diarrhea is among a disease which caused by multifactorial agents (viruses, bacteria, protozoa) [7]. Among these etiological agent of rotavirus infection only accounts around 27- 36% [9]. According few reports have been made under local conditions, the incidence and risk of diarrhea in calves varies from 20.05% to 52.51% [10-13]. Around 75% of early calf mortality in diary industry is caused by acute diarrhea in the early stage of calves [3].

Epidemiological study, conducting of the diagnostic tests, clinical observation and molecular characteristics of the causative agent is important for prevention and control of the infectious diseases. Clinically calf diarrhea is diagnosed as infectious enteritis but it is not easy to diagnosis at clinical and/or laboratory level, because of a number of reasons like the caused by multiple agents. In spite of the fact that, there is a number tests for diagnosis such as ELISA test, latex agglutination and RT-PCR. Among these methods, ELISA test was used detection of BRV in the feces of diarrheic calves [15].

Bovine rotavirus is the most known pathogens which leads acute diarrhea in cattle calves less than 28 days of age worldwide [16]. Acute diarrhea that caused by bovine rotavirus is the most common disease in both humans and animals. Even it has the zoonotic impact and economic impact [18]. Infection appears and spreads rapidly causing high destruction of the intestinal lining which leads in dehydration [19]. Genetic diversity of rotaviruses was created by genetic re-assortment which leads to evolution of the virus [20].

Rotavirus was widely studied in developed country and it distributed different area of the world [22]. In a number of studies bovine rotavirus prevalence was 20-60% in diarrheic samples have been stated [25]. In European countries rotavirus disease was extensively studied. In Asian countries such as Bangladesh, prevalence of rotavirus ranges from 0 to 7% [28]. In developing country like Ethiopia the prevalence of rotavirus was 16.7% [29].

Rotavirus in calves has not documented sufficient in Ethiopia. In sipe of the fact, only one studied found by Abraham., *et al.* (1992) which showed 16.7% of rotavirus in calves. But among children < 5 years of age rotavirus prevalence range from 18%-28% of diarrhea hospitalizations [30] and absence of such data or figure would be the cause of lack of the government have no any control strategy for rotavirus disease of calves via vaccination. Although livestock industry is a crucial component of agriculture-based economy of Ethiopia, new born animals suffer fairly higher production losses and mortality. Acute diarrheal disease in calves was commonly caused by rotavirus which leads in decrease productivity. Lack of such types of report on isolation and molecular detection of rotavirus in less than 30 day in calf in Ethiopia might aggravate the problem. Therefore, isolation and detection of the rotavirus by molecular is required for preparation a suitable control and preventive measure in the country.

Objective of the Study

Therefore, the objectives of present study were:

- To isolate rotavirus from calves and
- To detect the virus at molecular level in study area.

Material and Methods

The study area

The present study was conducted in certain site of central Oromia, Ethiopia (Bishoftu, Sebata, Holeta and Addis Ababa) from

November 2018 to May 2019 (See figure 1). Fecal samples were collected from all selected site of dairy farms. Both exotic and local breed were included. Bishoftu town is found in east Shewa Zone, Ethiopia, 45 km South-east of Addis Ababa. The area is placed at 9°N latitude and 40°E longitude at altitude of 1850m above sea level (Bishoftu City Administration Agricultural Desk, 2014). Sebeta town is found in Oromia region. The woreda is placed 25 km south west of Addis Ababa at an altitude of 1800-3385 m above sea level and at latitude and longitude of 8°55-8.917°N and 38°37-38.617°E respectively. Holeta city is placed in the central portion of the country, 31 km from west Addis Ababa. The area is confined between latitude 8° 53' 75" to 9° 14' North and longitude 38° 21' 40" to 38° 36' 14" East [31]. Addis Ababa, the capital city of Ethiopia, found between an elevation of 2300 m above sea level. It is located at 9°1′48″N latitude and 38°44′24″E longitude, geographical. It has a typical highland climate with temperature ranging from 11°C-24 °C. A mean annual rainfall of 1300 mm with bimodal distribution [32].

Figure 1: Study area map (By Author).

Study animals

The study was conducted in both apparently healthy calves and calves having clinical sign of diarrhea namely profuse watery diarrhea, systemic dehydration and depressed during investigation. Cow calves up to one month of age groups, all breed and sex reared under intensive management conditions were included in the study [33].

Sampling technique and sample size determination

Before the beginning of the actual study, preliminary data were obtained from the respective District Agricultural Office and dairy cooperatives to document the lists of dairy farms to guess the size of study population. The Study areas were purposively selected. Clinically diarrheic and non-diarrheic calves were sampled for detection of rotavirus. The sampling units were included zebu and exotic brad dairy calves aged less than 30 days.

To estimate the prevalence of bovine rotavirus in calves, sample size was determined by using simple random sampling method [34,35]:

$$n = \frac{1.96^2(p)\ (1-p)}{d^2}$$

p= Expected prevalence

d= Desired level of precision (5%)

n= Sample size.

Using expected rotavirus prevalence 16.7% in central Ethiopia (Abraham., *et al*. 1992), confidence level of 95% and required absolute precision of 5%; a total of 214 sample size was determined. However, a total of 245 calves were enrolled during the study period to enhance precision and to compare prevalence across different herd sizes.

Study design

A cross-sectional study was conducted in a number of dairy farms in study area. Data related with the calves was collected by interviewing farm owners and animal health workers of nominated study area. The collected data were recorded on collection sheet, and then calves were clinically examined for presence of diarrhea or not and fecal samples were collected for diagnostic testing as follows. During sampling all information related with the calf like (farm name, date of sampling, consistency of feces, age, breed) was collected for each calf on appropriate recording sheet.

Collection of fecal samples

Samples of fecal were collected in sterile collection tube after properly washing of the anal area [33]. Around 30 grams of fecal sample was collected directly from the rectum of calves. Collected samples were sited into universal ice box containing ice packs and transported to the virology laboratory at National Animal Health Diagnostic and Investigation Center, Sebeta and were stored at -80°C until processing.

Laboratory techniques

Detection of bovine rotavirus antigen by ELISA

Multiscreen Ag ELISA Calf digestive (BIO K 314/1, Belgium) is the type a sandwich ELISA capturing mixture of monoclonal antibodies (MAbs) against bovine rotavirus (BRV) was used to detect BRV antigen in the fecal sample. These antibodies react with the matching antigen in the fecal spaceman. The procedure of sandwich ELISA was conducted to detect rotavirus antigen in the fecal samples according to the manufacturer instruction (Kit reference BIO K 314/1, Belgium).

Extraction of rotavirus RNA

Rotavirus RNA was extracted from the fecal suspension using QIAamp viral RNA mini kit (Qiagen, UK) according the manufacturer's instructions. Around 1g of fecal specimen was added in to 1 ml of PBS. The mixture was vortexed vigorously for 40 seconds followed by centrifugation at 10,000 rpm for 5 minutes. All the supernatant (about 500 µl) was transferred to new tubes. The rest part of procedure was conducted following, according the manufacturer's instructions.

Viral isolation

Rotavirus positive sample by All ELISA test were taken forward for virus isolation. About 1 gm fecal sample was mixed with 9 ml antibiotic contain sterile PBS. Then centrifuged at 800 rpm for 15 minutes, the fecal suspension. The supernatant fluids were filtered through 0.45 µm membrane and filtrates solution were added with an equal volume of DMEM containing 5% fetal calf serum and 10 µg/ml crystalline trypsin and incubated at 37°C for 60 minutes. After incubation, one ml of the mixture was inoculated into the culture flasks with confluent monolayer of Madin Darby bovine kidney (MDBK) cell lines and kept for one hour cultivation to adsorption virus. After the adsorption at 37°C for one hour, the cells were washed 3 time with plain of DMEM maintenance media and incubated at 37°C in a humidified incubator having 5% CO_2. Monolayers were detected daily for development CPE for five days and viruses were sub-cultured blindly every two days after being subjected to 3 cycle of freezing and thawing. CPE was detected after 48 hours in positive sample and it was characterized by a destruction of the monolayer cell, cell rounding and infected cells were disrupted and detached from the flask. Cells displaying characteristic CPE were collected by freezing and thawing 3 times and centrifuged at 16,000 rpm for 20 minutes at 4°C for the removal of cell debris. The supernatant containing the virus was collected and stored at -80°C for further passages.

Reverse transcription polymerase chain reaction

The Reverse transcription polymerase chain reaction (RT-PCR) was made by using a RT-PCR Kit (QIAGEN) for the confirmation of bovine rotavirus. Primers for the amplification of gene segments VP4 were produced (See table 1) based on the earlier study [36]. For the desired amplification of VP4 gene (880 base pairs) of bovine rotavirus, optimization of PCR conditions was done by using the varying concentrations of dsRNA, primers and Taq polymerase in RT-PCR reaction mixture with total volume of 25 µl. Optimized reaction mixture for RT-PCR was dsRNA 2.5 µl, PCR buffer 2.5 µl, dNTPs 2.5 µl, MgC_2 2.5 µl, Forward Primer F (10 pmol) 3.0 µl, Reverse Primer 10µM (6µl), DNase/RNase free water 6 µl. Conditions for the amplification of VP4 gene of bovine rotavirus includes, before applying RT-PCR reaction, 2.5µl of viral dsRNA were denatured at 95°C for 5 minutes and chilled immediately for 5 minutes. Then, RT-PCR reaction was carried out with an initial reverse transcription (60 minutes at 42^0C), followed by activation PCR (at 94^0C for 15 minutes), 40 cycles of amplification (30 seconds at 94^0C, 45 seconds at 55^0C (annealing), and 45 seconds at 72^0C (extension)), with a final extension of 7 minutes at 72^0C. To examine the PCR product, agarose gel electrophoresis was conducted. The size of the PCR product for VP4 gene (880 bp) was illuminated in a gel documentation system.

Primer	Primer sequences	Size of amplicons	Melting Temp	Primer Length
VP4-F	TGGCTTCGCTCATT-TATAGACA	880 bp	54.2	22
VP4-R	ATTTCGGACCATT-TATAACC	880 bp	47.4	20

Table 1: Primer details.

Data management and statistical analysis

The collected data were entered in Microsoft Excel. Data of quantitative was coded and entered in a excel spread sheet and data was analyzed by the Stata 14 software.

Results

The current study, 245 (83 diarrheic and 162 non-diarrheic) fecal samples were collected from calves less than 1 month of age and then samples were screened for presence of rotavirus infection. From 83 diarrheic samples, 6 (7.2%) samples were found positive for rotavirus antigen by Ag-ELISA (See figure 2). But all

162 non-diarrheic samples were negative for rotavirus infection. 2.45% (6/245) were an overall prevalence of rotavirus in calves.

All the arrow with yellow color indicated the positive sample and non-arrow with yellow color positive control while arrow with white color was negative control. For the two groups of calves (diarrheic and non-diarrheic) were calculated separately, a prevalence of 7.23% (6/83) of rotavirus was observed in diarrheic calves and all none diarrheic samples were negative (0/162, Table 2). Antigen rotavirus positive samples in relation to their factors like ages, breed and sex of calves were displayed in table 2. The results showed that a prevalence for calves in the first week of ages was 4%. This indicated that calves of 1 - 2 weeks of age after born were more susceptible to rotavirus disease. But the observed rotavirus prevalence in different calve ages were not significant ($P > 0.05$).

Present study indicated that the males were more susceptible to rotavirus disease than female calves. A higher prevalence of 6.2% (5/81) was linked with male calves, while a prevalence of 0.6% (1/164) was recorded in female calves. The prevalence was significant for rotavirus between male and female calves (Fisher's exact = 7.0239; $p = 0.008$). A higher prevalence (4.9%) of rotavirus Ag was observed among calves fed colostrum's from 30 minutes to 2 hours compared to calves given colostrum's within 30 minutes of birth (0.8%). New born calves of cross breed cows were more susceptible to rotavirus infection than local breed (1.4%). The result showed a prevalence of cross breed was 2.9% and it not Significant ($P > 0.05$. The present study showed that the prevalence of rotavirus disease was higher in Sebeta (4%) as related with other selected area. The prevalence rotavirus was not significant among the selected areas ($P > 0.05$).

Calves floor area was concrete, more susceptible to rotavirus infection than the calves floor area was brick or muddy. The result indicated a prevalence of 2.8%, if the floor was concrete. The rotavirus prevalence was not significant ($P > 0.05$) between the calves' floor area. The result indicated that 6.9% calves separated immediately after birth from dam were found positive, whereas rotavirus was detected in 2.6% samples of calves separated greater than 24 hours after birth from dam (Table 2). The prevalence's were not significant ($P > 0.05$) for rotavirus between the times of separations of calves from dam. See table 2 for stratified prevalence of other variables.

In this study, MDBK cell line was used to isolate the virus from all samples of Ag-ELISA test positive samples. Out of 6 samples cultured on MDBK cell line, CPE was observed in 4 (66.7%) samples, while CPE was not observed on the remaining 2 samples (33.33%) even on third blind passage. In the first passage, infected cells did not show any CPE. But from second passage onwards the infected cells started showing characteristic CPE. At 24 hours post infection (p.i,) the infected cells became round and clumped. At 48 hours p.i, the cells were thin and round shaped. At 72 hours p.i, the cells became small and majority of monolayer detached (See figure 2).

Out of 6 positive samples 4 samples were screened by RT-PCR for molecular characterization due to the non-availability of sufficient quantity of fecal sample in the remaining samples. RT-PCR amplification of the 880 base pair fragments of the VP4 gene in the four samples that developed a CPE allowed to confirm the presence of rotaviruses (Figure 3). The Ag-ELISA negative calves' sample and negative on cell culture did not amplify by PCR. After RNA extraction and RT-PCR was done for amplification of VP4 gene. Out of the 4 fecal samples examined by RT-PCR technique, all samples were identified as positive (100%) and as expected, length of VP4 gene (880 bp) was generated for rotaviruses (See figure 3).

Discussions

Calves of newly born one is represent worldwide an important source in livestock production for meat or breeding i.e. replacement stock [37]. These industry faces many series disease problems like calf diarrhea, which usually affect it dramatically. Neonatal calf diarrhea is a most common disease affecting calves which cause to morbidity and mortality in calves. A crucial period for these calves is the first few days following birth. Domestic animals are the major source of income for developing country like Ethiopia. Fecal contamination plays an important role in transmission of rotavirus infection and the infection is widespread globally in cattle populations. For the effective control measures, prompt diagnosis of the disease is important [38].

In the present study, 6 of 245 fecal samples screened using Ag-ELISA was positive for rotaviral infection and all the positive animals were diarrheic calves while all the non-diarrheic calves were negative. The prevalence was found to be 7.23% in diarrheic calves. This result is in agreement with those reported by Prez., *et al.* (1998) in Costa Rica (7%), Duman and Aycan (2010) in Turkey (8.5%), Yilmaz (2016) in Turkey (8.92%), and Rajendran and Kang (2014) in India (5.5%). Higher prevalence rate of rotavirus have been reported from many countries including Abraham., *et al*. (1992) in Ethiopia (16.7%), Ammar., *et al.* (2014) in Algeria

Distributions of calf parameters	Level	Number of samples screened	Number of samples positive	Prevalence (%)	Fisher's exact test	P-value
Calves Health	Diarrheic	83	6	7.23	12.0048	0.001*
	Non-diarrheic	162	0	0		
Sex	Male	81	5	6.2	7.0239	0.008*
	Female	164	1	0.6		
Age	1st week	100	4	4	2.1263	0.547
	2nd week	106	2	1.8		
	3rd week	18	0	0		
	4th week	21	0	0		
Location	Bishoftu	60	2	3.3	1.3771	0.711
	Addis Ababa	123	3	2.4		
	Sebeta	25	1	4		
	Holeta	37	0	0		
Breed	Cross	174	5	2.9	0.0461	0.830
	Local	71	1	1.4		
Floor of the calf's area	Concrete	179	5	2.8	0.4364	0.804
	Brick	60	1	1.7		
	Muddy	6	0	0		
First colostrum feeding after birth	Within 30 minutes	132	1	0.8	4.3263	0.115
	Within 2 hours	103	5	4.9		
	Within 2-6 hours	10	0	0		
Separation of calves from dam	Immediately after birth	29	2	6.9	4.2418	0.120
	<24 hours	71	0	0		
	>24 hours	145	4	2.6		

Table 2: Distribution of bovine rotavirus in screened calves' samples.
Note: P value - Level of significance. Significant when P-value ≤ 0.05.

(14.63%), Kyle (2007) in Vietnam (15%), Pisanelli., *et al.* (2005) in Southern Italy (16.8%), Jindal., *et al.* (2016) in India (27.02%) and Uhde., *et al.* (2008) in Switzerland (58.7%). However, the result of this study is higher when compared to that reported by Fiedler., *et al.* (1982) in Oldenburg (1.96%). The discrepancy of results could be attributed to the age and the sample size difference. Prevalence of rotaviral infection varies depending on the country and region under study (Basera., *et al.* 2010; Radostitis., *et al.* 2007). All the 6 rotavirus positive samples were from diarrheic calves under the age of 4 weeks. Similar results were recorded by Sharma (2004) in bovine calves.

The current result could suggest that male calves (6.2%) were higher susceptible for rotavirus infection than female calves (0.6%). Other studies like Dash., *et al.* (2011) and Sharma (2004) also reported higher susceptibility of male bovine calves (20.37%) and (42.85%) in comparison to female calves (12.76%) and (28.2%), respectively. The possible justification for this could be due to immune system in that Odde (1988) reported higher anti-rotavirus IgG concentrations for female calves compared to male calves. It could be due to the managemental practices as in most of the dairy farm's female calves are better looked after than male calves. Previ-

Figure 2: Characteristic CPE of rotavirus inoculated on MDBK cell line.
Shows rounding and cell detachment from the flask and lysis of cells (indicated by arrow) against respective controls (A).

Figure 3: cDNA band corresponding to rotavirus VP4 genes detected on agarose gel.
From left to right: Lane M, Molecular weight markers; Lane P, is positive control; Lane N, is negative control; Lane 1, 2, 3, 4, is positive sample.

ously, Ammar., *et al.* (2014) and Dash., *et al.* (2011) also reported higher susceptibility of male bovine calves in comparison to female calves against rotavirus infection. In line with this, Clement., *et al*. (1995) noticed that males calves were double susceptible to diarrhea as compared to female calves.

Age wise, the susceptibility of newborn calves of 1st week up to 2nd weeks of age to rotavirus infection were more than older calves. The occurrence of rotavirus in the fecal samples of diarrheic calves was found to decrease with increase in the age of the calves. The finding of the present study is in agreement with the earlier workers reported by Abraham., *et al.* (1992), Jindal., *et al.* (2000) and Singh., *et al.* (2009), higher occurrence of rotavirus infection in diarrheic calves were mainly restricted to the first 2 weeks of life. Maximum prevalence of rotavirus diarrhea was observed in 5 - 21 days old calves [37]. The 2 weeks old calves were the most susceptible to rotavirus infections, which may be due to decreasing of passive immunity and the absence of the natural resistance against infection. The 3-weeks-old calves are characterized by absence of rotavirus, this may be highlighted by an increased natural resistance against infection [33].

The results showed that the prevalence was slightly higher in the Sebeta (4%) than in Bishoftu (3.3%) and Addis Ababa (2.4%) towns. This could be attributed to sample size of the areas and presence of higher number of factories in the near farm that can be a source of contamination for animals. In the present study higher prevalence was recorded in crossbred calves (4.2%) than local calves (2.8%) that similar to previous report of Sharma (2013).

In the present study, viral growth in cell culture was assessed by examining inoculated cells for CPE. Out of 6 samples, only 4 of the 6 Ag-ELISA positive samples established infection in MDBK cells as determined by production of characteristic CPE on the second passage and it continued up to third passages. The CPE observed were characterized by rounding, detachment as well as destruction of mono-layer cell. The CPE produced in this study were in agreement with previous reports (Saravanan., *et al*. 2006).

The RT-PCR technique confirmed the presence of rotaviruses in fecal samples that were previously diagnosed by ELISA and growth in cell culture. The RT-PCR-based genotyping method used was further confirmed to be a useful epidemiological tool and to determine the presence of rotavirus nucleic acid by using specific generic primers for VP4 genetic regions in feces samples [59]. This study is comparable with previous reports (Reidy., *et al*. 2006).

Conclusion and Recommendations

The present investigation was undertaken to investigate the prevalence of rotavirus status in cow calves lessen than one month of age at different selected farms in central Oromia. The effect of age, sex, breed and house floor of calves on prevalence of diarrhea was also studied. Using Ag-ELISA, 6 samples were identified as positive, and all of the isolates were obtained from diarrheic calves in the 1st and 2nd weeks of age. The result indicated that there is

an association between rotavirus detection and sex of calves in that the prevalence of rotavirus is higher in male calves than female calves. In addition, the prevalence is higher in calves kept in concrete floor, this fed colostrum later (within 2 hours) as well as in local bred calves and in calves separated from their dams immediately after birth. When the 6 Ag-ELISA positive samples were cultured on MDBK cell line, only 4 samples showed cytopathic effect (CPE). Observational study and questionnaire survey also indicated that only awareness of the advantage of colostrum feeding is not enough, but also times of colostrum administration to neonate calves are crucial for the ultimate development of immune status against pathogens including rotavirus infection. Calving areas should have well-drained grass lots or pastures visible from the barn area and calving areas should be selected or landscaped to allow for adequate drainage. Enteric disease like rotavirus infection is a vital health problem in calves that interrupts production benefits with reduced weight gain and increased mortality, and the virus potential for its zoonotic spread, it is imperative to determine the disease burden and responsible risk factors. This is very useful to execute effective preventive measures such as practicing early colostrum feeding in newborn calves, vaccination in dams and improving livestock management. So, this study shows that there is rotavirus infection in diarrheic calves in Ethiopia. An evaluation of data leads to the conclusion that vaccination program needs to be implemented to bring this disease under control.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Consent

Verbal consent was also obtained from the farm managers to take samples from their cattle and for further research use of the samples.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Umer S contributed to conception of the research idea, designing and data collection, data analysis, interpretation of data, and writing and editing of the manuscript. Fufa D contributed to the study concept, interpretation of data, and editing or reviewing of the manuscript. Munera A contributed to conception of the research idea. Asamino T contributed to the data analysis, interpretation of data, and writing and editing of the manuscript. Melaku S contributed by conducting laboratory Investigation. All authors read and approved the final manuscript.

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