Seroprevalence of bovine viral diarrhea virus and detection of persistently infected (PI) animals in dairy farms of Holeta, central Ethiopia

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Abstract

Bovine viral diarrhea virus (BVDV) is one of the most important pathogens of the reproductive systems that have a significant socioeconomic impact on the dairy industry. A cross-sectional study was conducted on 17 randomly selected farms out of 133 registered dairy farms in Holeta, a town in the Walmera district of Ethiopia. The study aimed to detect BVDV antigen-positive animals and investigate the seroprevalence of BVDV infection. A total of 337 serum samples were collected from selected farms with no history of BVDV vaccination. The indirect enzyme-linked immunosorbent assay (I-ELISA) test was the screening test used to detect antibodies against BVDV. In contrast, antigen-capture ELISA was used for the detection of BVDV antigens in seronegative animals. A logistic regression model was used to assess the association between potential predictors and BVDV exposure. The overall animal level seroprevalence was 15.4%, and 64.7% of the herds had at least one seropositive animal. From 285 seronegative animals, one animal (0.4%) was found positive for BVDV antigen. The same animal was found positive in a double check 21 days later. In this study, cows with a history of abortion (OR = 6.3; 95% CI: 1.61 -13.1), history of repeated breeding (OR = 7; 95% CI: 2.5 - 14.3), animals managed intensively (OR = 4.6; 95% CI: 1.6 - 13.0) and multiparous cows (OR = 3.6; 95% CI: 1.5 - 8.9) had higher proportion of seroreactors in their respective comparison category ($p<0.05$). Besides, cows with a history of congenital defective calve birth (OR = 15.2; 95% CI: 3.2 - 73.6), adult age groups (OR = 2.9; 95% CI: 1.0-7.9), and cows bred both artificially and natural mating (OR = 4.6; 95% CI: 1.7 - 12.6) were statistically associated with BVDV seropositivity ($p<0.05$). In conclusion, this report demonstrated the presence of persistently infected (PI) dairy cattle in Ethiopia. Thus, the findings warrant the need for

immediate control intervention that involves both screening and culling of PI animals and vaccination.

Keywords: BVDV; Dairy Cattle; ELISA; Seroepidemiology; Holeta; Ethiopia.

Introduction

Efficient reproduction in cattle is critical for the economic success of the dairy industry. Especially in developing nations like Ethiopia, reproductive inefficiency significantly affects the livelihoods of livestock owners. The problem has been linked to multiple infectious and non-infectious causes. Among these, bovine viral diarrhea virus (BVDV) is one of the most important infectious pathogens that impose a significant economic impact on the dairy industry worldwide (Albrecht *et al.*, 2021).

A single-stranded RNA virus causes bovine viral diarrhea in the genus *Pestivirus* of the *Flaviviridae* family (Brodersen, 2014). The virus has two genetically distinct genotypes (BVDV-1 and BVDV-2) and two biotypes (cytopathic and non-cytopathic). Infection with BVDV leads to significant economic losses, both directly and indirectly. Directly, it affects the reproductive performance of dairy herds, causing a decline in conception rates, increased rates of abortion, stillbirths, the birth of weak calves, teratogenesis, and reduced milk yield. Indirectly, the expenses incurred for implementing control programs further contribute to the economic burden (Lindberg, 2003; Pinior *et al.*, 2017). Besides, infection of cattle during the early gestation period may lead to the birth of persistently infected (PI) calves that have great epidemiological relevance in the dynamics of BVD infections (Albrecht *et al.*, 2021).

Bovine viral diarrhea virus survival in the cattle population mainly depends on the characteristics of PI cattle. Persistent infection arises from the unique ability of the BVDV to survive by inducing immune tolerance in the bovine fetus through evasion of both innate and acquired immunity in utero (Brodersen, 2014). The main transmission route in infected herds is direct contact with PI animals (Lindberg, 2003). Viral transmission can also occur through the placenta to the developing fetus (Brownlie, 1987).

Reproductive diseases in dairy cattle can be prevented by vaccination and the implementation of biosecurity measures. Vaccination against BVDV improved

reproductive efficiency parameters in dairy herds (Pereira *et al*., 2013). However, vaccines against BVDV are not available in Ethiopia. Hence, identification of the PI cattle is crucial for the implementation of management practices that decrease the risk of exposure of susceptible dairy cattle. BVDV control and eradication have primarily focused on identifying and removing the PI cattle from the herd. Although PI cattle are the primary reservoir of the virus, their identification and elimination from susceptible cattle populations can be costly in the form of diagnostic testing since less than 1% of the cattle population is PI with BVDV (Newcomer *et al*., 2017; Peddireddi *et al*., 2018).

In Ethiopia, reproductive abnormalities, including repeat breeding, abortion, stillbirth, fetal mummification, and congenital abnormalities, have been reported in previous studies, and the presence of antibodies against BVDV in dairy cattle has been recorded with variable prevalence (Nigussie *et al*., 2010; Asmare *et al*., 2013; Aragaw *et al*., 2018). However, many epidemiological data gaps still exist, and no information is available on the status of PI cattle. Thus, more investigations need to be done to identify the PI cattle from dairy herds and better understand the epidemiology of BVDV in the different geographical settings of the dairy cattle population, which could help to formulate strategies to mitigate the impact of BVDV on the production and reproduction of dairy cattle in Ethiopia. Hence, the present study aimed to investigate the seroprevalence of BVDV and detect BVDV antigens in dairy cattle in Holeta town.

Materials and methods

Study area and population

The study was conducted in Holeta, a town in the Walmera District, West Shoa Zone, and Oromia regional state (Figure 1). Holeta, a town in central Ethiopia, is known for its abundance of dairy farms and the long tradition of keeping improved dairy cattle. Smallholder dairy farms are major employment areas and sources of income for youths and women in the town. Cattle are the dominant livestock in the area. Holeta town has 133 registered dairy farms, according to the Walemera District Livestock Development and Fishery Office (WDLDFO, 2019).

The target population was Holstein-Friesian crossbreeds with no history of vaccination, breeding females, and reared in smallholder, medium, and large commercial dairy farms.

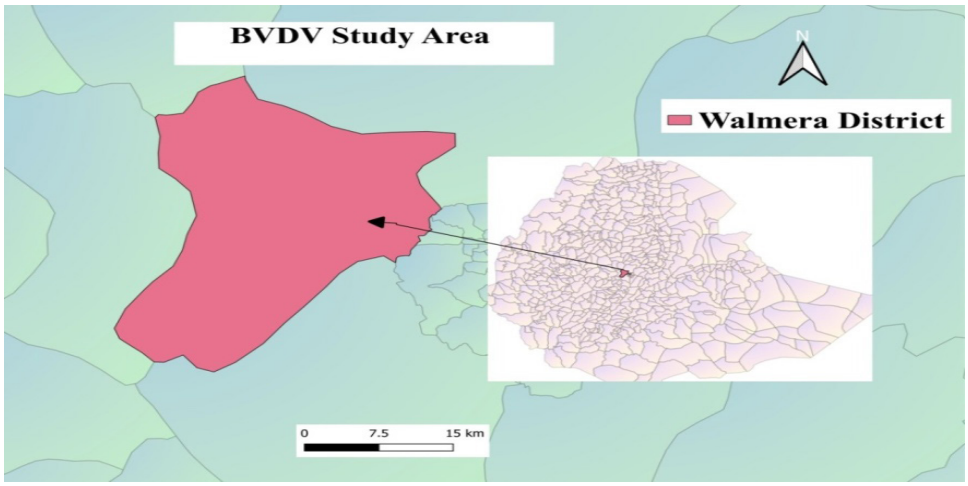

Figure1. Map of Ethiopia that shows the study area.

Study design, sampling, and sample size

A cross-sectional study was conducted on 17 randomly selected farms out of the 133 registered dairy farms in Holeta. Animal biodata and management-related factors were recorded to identify the potential risk factors associated with BVDV, such as age, herd size, farming systems (intensive and semi-intensive), parity [nulliparous (no calving), primiparous (given calving once), and multiparous (given two or more calving)], animal husbandry and housing practices, the structure of the dairy farm, composition of dairy herds, and breeding system (using artificial insemination (AI) and/or bull). Herds were classified into small (1-15 animals), medium (15-25 animals), and large (above 25 animals) herds. The age was grouped into six months to < 1 year, 1-2 years, and above two years.

Simple random sampling techniques were used to select 17 farms from 133 registered dairy farms in the town. The minimum sample size was estimated based on Thrusfield (2005). The predetermined parameters were an expected prevalence of 32.6% (Aragaw *et al.,* 2018), a 95% level of confidence, and 5% absolute precision. Accordingly, a total of 337 blood samples were collected.

All seronegative serum samples were subjected to antigen capture ELISA to detect PI animals. The positive animals were rechecked for their status after 21 days.

Blood sample collection

Ten ml of blood samples were collected using a plain vacutainer tube from the jugular veins of 337 randomly selected dairy cattle. The blood samples were labeled and allowed to stand overnight, and then the serum was decanted into cryovials. The collected serum samples were transported to the Animal Health Institute (AHI) in an icebox and kept at -20 °C until tested.

Laboratory tests

Indirect ELISA assay targeting antibodies

BVDV-specific antibodies were detected using indirect enzyme-linked Immunosorbent Assay (iELISA) (IDEXX, France) that detects antibodies against p80 protein of BVDV with a diagnostic sensitivity of 97.6 % and specificity of 97.27 % according to the information of the manufacturer's validation data report. BioTek EL800 Microplate Reader was used to measure the optical density (OD) of each sample and control at 450nm. The results were interpreted based on the sample-to-positive control OD ratio (S/P). Samples with an S/P ratio greater than or equal to 50 % were considered negative, whereas samples with an S/N ratio less than or equal to 40 % were considered positive; samples with an S/N ratio between 40% and 50 % were considered doubtful.

Antigen capture ELISA assay

Negative serum samples in the iELISA were subjected to antigen capture ELISA assay. The detection of BVDV antigen in serum samples was performed using the manufacturer's instruction (BVDV Ag/Serum Plus, IDEXX, Switzerland). This test kit has a diagnostic specificity of 100% (95% CI: 95.69 - 100) and a sensitivity of 100% (95% CI: 90.5 – 100). The test protocol includes adding a positive and negative control to each plate for every run of the assay. The sample/positive control ratio (S/P) was calculated using the following formula, and the sample was considered BVDV positive if the S/P ratio was ≥ 30.

S/P = (OD of the sample-OD of the negative control)/(mean OD of the positive control-OD of the negative control) x 100

Data analysis

Data were recorded and coded using Microsoft Excel for Windows 2010 and transferred to STATA13.1 (Stata Corp. College Station, Texas) software for analysis. The data were then summarized using descriptive statistics. A univariable logistic regression analysis was used to assess the unconditional association of prevalence with individual risk factors for BVDV exposure. Then, the independent variables were checked for multicollinearity using Variance Inflation Factors (VIF) and tolerance value (Dohoo *et al.*, 2003). Variables with p-value ≤ 0.25 in the univariable logistic regression analysis with no multicollinearity were entered into the multivariable logistic regression model. The forward elimination procedure was used to eliminate the factors that were not significant ($p > 0.05$). The model was finally assessed using the Hosmer-Lemeshow test for goodness-of-fit and the receiver-operating curve (ROC) for reliability. The model with a value of the area under the curve (AUC) of the ROC greater than 0.7 was accepted. In all analyses, the confidence level of 95% and $p < 0.05$ was set as statistically significant.

Results

Out of the total of 337 serum samples tested, 15.4% (95% CI: 11.7 – 19.7%) were positive for antibodies against BVDV. Similarly, from a total of 285 seronegative animals subjected to antigen-capture ELISA, one animal (0.4%) was found positive for BVDV antigen (Table 1). The same animal was found positive in a double check 21 days later. Similarly, the overall seroprevalence of BVDV in herds with at least one positive animal was 64.7% (95% CI: 38.3 - 85.8%).

Table 1. Animal and herd-level prevalence estimate of BVDV in Holeta dairy farms

Variable	No examined	No. of positives	Prevalence (95% CI)
Individual animal level	337	52	15.4 (11.7 – 19.7)
Herd level	17	11	64.7 (38.3 - 85.8)

Cl: Confidence interval

BVDV seroprevalence and associated risk factors

Results from the initial univariable logistic analysis are given in Table 2. The risk factors such as age, herd size, parity, and farming systems were significantly ($p < 0.05$) associated with BVDV seropositivity. In the next multivariable logistic regression model, significant differences in seroprevalence were observed in age, farming system, parity, and breeding system (Table 4). In this model, a significantly higher seroprevalence was observed in animals greater than two years old (OR = 2.9; 95% CI: 1.0 – 7.9; $p = 0.043$), multiparous cows (OR = 3.6; 95% CI: 1.46- 8.59; $p \leq 0.001$), in intensive farms (OR = 4.6; 95% CI: 1.6 - 13.1; $p = 0.004$) and farms using both bulls and AI for breeding (OR = 4.57; 95% CI: 1.7- 12.6; $p = 0.003$) (Table 4).

Table 2. Univariable logistic regression analysis for potential risk factors associated with BVDV exposure status in dairy cattle.

Variables	No. examined	Prevalence (%)	COR (95% CI)	p-value
Age				
Six months - <1 year	95	7.4	Ref	
1 -2 years	56	3.6	0.5 (0.1 - 2.3)	0.351
> 2 years	186	23.1	3.8 (1.6 - 8.8)	0.002
Herd Size				
Small	69	8.7	Ref	
Medium	107	6.5	0.7 (0.2 - 2.3)	0.595
Large	161	24.2	3.4 (1.4 - 8.4)	0.009
Parity				
Nulliparous	154	6.5	Ref	
Primiparous	63	20.6	3.6 (1.4 - 9.3)	0.008
Multiparous	120	24.2	4.4 (1.9 - 10.1)	≤ 0.001
Farming system				
Semi-intensive	197	7.61	Ref	
Intensive	140	26.4	4.4 (2.3 - 8.3)	≤ 0.001
Breeding System				
AI	79	8.9	Ref	
AI and Bull	258	17.4	2.17 (0.9 - 5.0)	0.070

Ref: reference; COR: Crude Odds ratio; CI: Confidence interval

Association of BVDV seroprevalence and reproductive problems

Univariable logistic regression analysis showed that cattle with a history of abortion, repeat breeding, and congenital defects were significantly ($p < 0.05$) associated with BVDV seropositivity, as shown in Table 3. A multivariable logistic regression model was fitted to predict the association of BVDV seropositivity with a history of abortion, repeat breeding, and congenital defects. Within this model, the risk of being BVDV seropositive was six times higher in cows with a history of abortion (OR = 6.3; 95% CI: 2.4 - 16.7; $p \leq 0.001$) compared to cows without a history of abortion. Similarly, the risk of being BVDV seropositive was six times higher in cows with a history of repeat breeding (OR = 5.9; 95% CI: 2.5 – 14.3; $p \leq 0.001$) compared to cows with no history of repeat breeding. The risk of being BVDV seropositive was 15 times higher in cows with a history of congenital defects (OR =15.2; 95%CI: 3.2 – 73.6; $p \leq 0.001$) compared to calves without a history of congenital problems (Table 5).

Table 3. Univariable logistic regression analysis of reproductive problems with BVDV exposure status in dairy cattle.

Variables	No. of tested animals	Prevalence (%)	COR (95% CI)	p-value
Abortion history				
No	312	12.5	Ref	
Yes	25	52.0	7.0 (2.9 - 16.6)	≤ 0.001
History of Repeat Breeding				
No	303	11.9	Ref	
Yes	34	50.0	6.1 (2.9 - 13.1)	≤ 0.001
History of Congenital Defects				
No	325	14.5	Ref	
Yes	12	41.7	4.2 (1.3 - 13.9)	0.017

Ref: reference; COR: Crude Odds ratio; CI: Confidence interval

Table 4. Multivariable logistic regression for the potential risk factors with BVDV exposure status in dairy cattle.

Variables	AOR (95% Confidence interval)	P-value
Age		
≥ 2 years	2.9 (1.0- 7.9)	0.043
1 -2 years	0.3 (0.6 - 1.8)	0.190
Herd size		
Medium	1.1 (0.2- 5.9)	0.874
Large	3.5 (0.5- 22.7)	0.191
Farming system		
Intensive	4.6 (1.6 - 13.1)	0.004
Parity		
Multiparous	3.6 (1.56- 8.6)	0.005
Primiparous	1.6 (0.5- 4.7)	0.408
Breeding system		
AI and Bull	4.57 (1.75- 12.69)	0.003

Table 5. Multivariate logistic regression analysis of reproductive disorders with BVDV exposure status in dairy cattle.

Variables	AOR (95% Confidence interval)	P-value
Abortion history		
Yes	6.3 (2.4 - 16.7)	≤ 0.001
History of Repeat Breeding		
Yes	5.9 (2.59 - 14.3)	≤ 0.001
History of Congenital Defects		
Yes	15.2 (3.2 - 73.6)	0.001

Discussion

In this study, 15.4% animal-level seroprevalence of BVDV was recorded. The animal level seroprevalence of this study was comparable with previous reports of Nigussie et al. (2010), with an overall seroprevalence of 11.5% in Jimma, Shoa, and South Shoa zones of Ethiopia, and Asmare et al. (2013) with a seroprevalence of 11.7% in dairy farms of southern Ethiopia. This study was also in agreement with the previous reports in Sudan (10.7%) (Saeed et al., 2015), Egypt (10.4%) (Soltan et al., 2015), and Kenya (19.8%) (Callaby et al.,

2016). On the other hand, the current findings were lower than the findings reported in Ethiopia (32.6%) (Aragaw et al., 2018), Mexico (78.8%) (Montiel, 2019), Iran (77.9%) (Shirvani et al., 2012), and Nigeria (64.4%) (Bello et al., 2016). In the current study, 64.7% herd-level seroprevalence of BVDV antibodies was reported. This finding is in agreement with a herd prevalence of 69.8% in Ethiopia (Aragaw et al., 2018), 65.5% in Brazil (Fernandes et al., 2016), 66% in Great Britain (Velasova et al., 2017), and 69% in Colombia (Ortega et al., 2020). The difference in animal and herd level seroprevalence might be attributed to differences in animal management systems, the diagnostic test used, sample size, study design, and environmental (agroecology) conditions (Houe, 1999; Saa et al., 2012; Fernandes et al., 2016).

In this study, a significantly higher seroprevalence was observed in the intensive farming system compared to the semi-intensive farming system. This might be due to the high contact rate in intensively managed herds that facilitate virus transmission. Van Campen reported that herd density is a significant predictor of the prevalence of BVDV (Van Campen, 2010).

In the current study, the seroprevalence of BVDV antibodies was significantly associated with dairy cows with a history of abortions. The risk of being BVDV seropositive was six times higher in cows with a history of abortion (OR = 6.3; 95% CI: 2.4- 16.7; $p \leq 0.001$) compared to cows without a history of abortion. This suggests that there is an association between abortion and BVDV infection. This result was in agreement with other studies that reported a higher seroprevalence of BVDV antibodies in cows with a history of abortions (Asmare *et al*., 2013; Derdour *et al*., 2017; Tadesse *et al*., 2019; Thapa *et al*., 2019).

In this study, the risk of being BVDV seropositive was six times higher in cows with a history of repeat breeding (OR = 5.9; 95% CI: 2.49 – 14.30; $p \leq 0.001$) compared to cows with no history of repeat breeding. This suggests that BVDV might be responsible for repeat breeding in dairy cows in Ethiopia. This result is in agreement with previous findings in Ethiopia (Asmare *et al*., 2013; Nigussie *et al*., 2010). Reduced conception rates, early embryonic deaths, abortions, congenital defects, and the birth of weak calves have been associated with BVDV infection in dairy cattle (Kaiser *et al*., 2013).

In this study, BVDV antigen was detected at a prevalence of 0.35%. To the authors' knowledge, this is the first report demonstrating BVDV antigen in dairy cattle in Ethiopia. This study indicates the presence of PI animals in

Ethiopia's dairy herds. A single PI animal can serve as a potential source of infection for the entire herd and contribute to the spread of the virus. PI animals are critical virus transmitters since they continuously shed a large amount of viruses (Lindberg, 2003). A previous study showed that BVDV PI prevalence at the animal level ranged from low (≤ 0.8%) in Europe, North America, and Australia to medium (>0.8 to 1.6%) in East Asia, and high (>1.6%) in West Asia (Scharnböck *et al*., 2018). At present, there is no BVDV vaccine available in Ethiopia, and no control strategies have been implemented. Scharnböck *et al.* (2018) identified the highest PI prevalence at the animal level in countries that had failed to implement any BVDV control and/or eradication programs, including vaccination. Thus, improving the overall biosecurity measures of the farms and early detection of PI animals to eliminate them from the herd is crucial.

Conclusions

In this study, the reported animal level prevalence is moderate, but the herd level prevalence is apparently high. To the author's knowledge, this is also the first report demonstrating PI animals in dairy cattle in Ethiopia. Multiparous cows, cattle reared in an intensive production system, and older age significantly increase the risk of BVDV infection. Moreover, specific reproductive abnormalities such as abortions, congenital defects, and repeat breeding were associated with seropositivity of BVDV. Thus, there is a need for control intervention focusing on screening and culling of PI animals and vaccination to protect the herd, at least in the area.

Data availability

The data collected and used to support this article can be offered by the first or corresponding author upon request.

Competing interest

The authors declare that they have no competing interests.

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tion, design, and interpretation of the study, data collection, analysis, and interpretation of the data reported in this manuscript.

Ethical considerations

Ethical approval for this study was granted by the animal research ethical review committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University (reference number: VM/ERC/19513/2021). Animals were handled following the best veterinary care guidelines. Before conducting the research, animal owners were informed about the objectives and the benefits of the study, and they gave consent for their animal's inclusion in the study. The consent obtained from animal owners was verbal because owners are unable to write and read. Consent was obtained in the presence of a third independent party and approved by the College of Veterinary Medicine and Agriculture of Addis Ababa University Ethics Committee.

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