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Research Article

Bovine Herpesvirus-1 Seroprevalence and its Associated Risk Factors in Dairy Farms in Holeta Town, Oromia Region, Ethiopia

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Abstract

Background: Bovine herpesvirus-1 is responsible for the development of severe respiratory infections, and commonly causes infectious bovine rhinotracheitis in cattle. However, infectious bovine rhinotracheitis has not been brought under control in Ethiopia because of a lack of well-organized disease impact assessment, the absence of identification of an associated risk factor, and the limitation of causative agent identification. Hence, this study aimed to investigate the seroprevalence of bovine herpesvirus-1 and its associated risk factors in dairy animals in the study area.

Methods: A cross-sectional study was conducted to estimate the seroprevalence of bovine herpesvirus 1 infection from March 2021 to November 2021 in selected dairy farms in Holeta Town, Ethiopia. A total of 390 serum samples were collected from 16 randomly selected dairy herds and tested using indirect antibody detection indirect-ELISA. Both Pearson's chi-square and logistic regression tests were used to compare the associations between BoHV-1 sero-positivity and postulated risk factors.

Results: The overall seroprevalence of the disease in the study area was 32.6% (n = 127, 95%CI: 28.1% - 37.4%) and 93.75% (n = 15) at animal and herd levels, respectively. Based on a multivariable logistic regression model, respiratory problems (AOR = 18.79, 95%CI: 5.23 – 67.55; ρ <0.001), and abortion (AOR = 0.30, 95%CI: 0.10 – 0.90; ρ = 0.033) were significantly associated with infectious bovine rhinotracheitis in cattle in the study area.

Conclusion: It was concluded that bovine herpesvirus-1 infection circulates in almost all dairy farms. Thus, virus isolation and characterization are mandatory. Hence, effective prevention and control strategies must be implemented to counter losses incurred by the virus.

Keywords: Bovine Herpesvirus 1; Dairy Farms; Holeta; Risk Factors; Seroprevalence

Abbreviations

AOR: Adjusted Odds Ratio; AHI: Animal Health Institute; AI: Artificial Insemination; BCS: Body Condition Score; BoHV-1: Bovine Herpesvirus Type 1; BPI-3V: Bovine Parainfluenza-3 Virus; BRD: Bovine Respiratory Disease; BRSV: Bovine Respiratory Syncytial Virus; BVDV: Bovine Viral Diarrhea Virus; CI: Confidence Interval; COR: Crude Odds Ratio; DNA: Deoxyribonucleic Acid; dsDNA: Double-Stranded DNA; ELISA: Enzyme-Linked Immunosorbent Assay; HF: Holstein Frisian; IBR: Infectious Bovine Rhinotracheitis; TG: Trigeminal Ganglion; UL: Unique Long; US: Unique Short; CD: Chala Dima; KA: Kebede Abdisa; DZ: Demeke Zewde

Introduction

Livestock plays a critical role in the welfare of rural populations of the world and its economy. Different respiratory and reproductive disorders in dairy animals due to different etiological agents have led to significant economic losses in dairy animals [1]. Among these agents that cause respiratory diseases, bovine respiratory disease (BRD) includes bovine viral diarrhea virus (BVDV), bovine herpesvirus-1 (BoHV-1), bovine parainfluenza-3 virus (BPI-3V), and bovine respiratory syncytial virus (BRSV), which are commonly known agents [2,3]. Of the above-mentioned viral agents, BoHV-1 is one of the most widespread respiratory and reproductive viral diseases in bovines globally [4].

Bovine herpesvirus-1 is responsible for the development of severe respiratory infections in bovines. The virus causes Infectious Bovine Rhinotracheitis (IBR) in high-producing cattle, infectious pustular vulvovaginitis (IPV) in cows, and Infectious Pustular Balanoposthitis (IPB) in bulls [5]. *BoHV-1* belongs to the order *Herpesvirales,* the *Herpesviridae* family in the *Alphaherpesvirinae* subfamily [6], in the genus *Varicellovirus* [7], and is an economically important pathogen that causes IBR in cattle [8].

The BoHV-1 infection has a global distribution [9] and shows significant regional variation in incidence and prevalence [5]. BoHV-1 has been known to cause disease in cattle for many years, and the first report of the disease believed to be caused by BoHV-1 came from the United States in the early 1950s [10]. This virus is one of the most important viral infections of buffaloes as well all over the world [11], except in BoHV-1 free countries. Although its mortality is low, the disease has a severe impact on growth, milk production, and international livestock trade, causing it to be included in a European list of diseases that may require control and eradication programs [12]. In Ethiopia, four preliminary surveys have been conducted in limited geographic areas, confirming the presence of BoHV-1 infection in cattle. 41.8% in Harar and Sidamo provinces [13], 67% in Gobe and Ghibe [14], 41% in Central Ethiopia [15], and 25.6% in Dessie and Kombolcha towns in the South Wollo Zone of the Amhara Region [16], respectively.

The genome of BoHV-1 is large, linear, enveloped, and double-stranded DNA (dsDNA) [17], which consists of two segments; unique long (UL) and unique short (US), and each segment encodes some glycoproteins. These glycoproteins are located in the envelope on the surface of the virions. They play an important role in virus-cell interaction [18] and have a vital role in the pathogenesis and development of immunity [19]. The genome belongs to the D group in the classification and consists of a long dsDNA molecule that encodes 70 proteins, of which 33 are structural and 15 are non-structural [20]. Moreover, the BoHV-1 viral genome consists of 12 enveloped glycoproteins, namely; gB, gC, gD, gE, gG, gH, gI, gK, gL, gM, gN, and Us9. Of these, the former ten proteins are glycosylated and the latter two are non-glycosylated. The glycoproteins; gB, gC, and gD proteins, are all considered major proteins. The total molecular size of the *BoHV-1* genome is approximately 135-140 kbp. Generally, proteins of the subfamily *Alphaherpesvirinae* are highly essential for host entry, pathogenesis, and immunity development [21].

The transmission of IBR is mainly based on the rich viral sources of the infected materials. Nasal exudates, coughed-out droplets, genital secretions, semen, foetal fluids, and tissues are considered as potential viral materials for transmission [6,22]. Direct nose-to-nose contact is the main mode of transmission that occurs between infected and susceptible cattle [6]. In addition, BoHV-1 can survive at +4oC for up to a month [23], in feed for more than 30 days [22], and in frozen semen stored in liquid nitrogen for up to a year [6,22]. The virus is mechanically transmitted between bulls during semen collection. Venereal transmission has become the method of spread for genital diseases, and infected semen can also act as a source of infection for a susceptible female by artificial insemination (AI) and often is the main route of infection of the virus, causing IPV in cows [24].

Despite the presence of apparent immunity, the virus remains dormant in the affected cattle's trigeminal ganglion (TG), and when they are stressed for various reasons, they shed the virus into the environment and become a source of infection for other susceptible cattle. Latently infected cattle are considered carriers of the virus throughout their life spans [4]. It could be due to the immune evasion mechanism and reactivation of the virus following stress. Various intrinsic and extrinsic factors influence the prevalence of infection among cattle [5]. *Infected animals with BoHV-1 develop a variety of mild to severe clinical syndromes, including; rhinotracheitis, vaginitis, balanoposthitis, abortion, encephalitis, conjunctivitis, and enteritis, as well as decreased milk production and weight gain* [25] with nasal discharge, cough, and lachrymal discharge with or without mild diarrhea [26].

In the milieu of animal health control and disease prevention, it is fundamental to have access to efficient diagnostic tests capable of detecting early disease outbreaks and guaranteeing the absence of disease within large territorial extensions [27]. Therefore, sequencing of the viral whole genome is necessary to differentiate the BoHV-1 field strain from the vaccine strain based on single nucleotide polymorphisms (SNPs) [28]. It is also required for disease prevention and control approaches if a viral marker vaccine is not available.

In Ethiopia, of the several diseases that are known, IBR is the one that needs particular emphasis due to its economic impact, causing abortion in pregnant animals and a reduction in the production of cattle. In an intensive production system using AI service, disease transmission might be extended unless strict diagnostic service is applied. Unlike in most developed countries, IBR has not been brought under control in Ethiopia due to a lack of well-organized disease impact assessment, mismanagement of animal quarantine, unrestricted animal movement across the border, a gap in awareness creation, the lack of identification of a risk factor, and the limitation of causative agent identification and extended researches. Hence, this study was aimed at estimating the number of seropositive dairy animals for BoHV-1 infection and its associated risk factors in Holeta dairy farms.

Materials and Methods

Description of the study area

The study location was selected deliberately based on the information from local farmers that have a long history of animal husbandry and the Welmera district is known for providing Holstein Frisian (HF) and cross-bread cattle to others. Holeta town is found in the Welmera district, in the Oromia Special Zone surrounding Addis Ababa. The town is located 40km west of Addis Ababa at 9°30'N and 38°30'E with an altitude ranging from 2,300 – 3,800 meters above sea level. The area is classified as a temperate highland, with an annual rainfall of about 1,650 mm, according to a report by [29]. The mean annual minimum and maximum temperatures are 8°C and 19°C, respectively. Farmers in the Welmera district have an estimated total of 195,778 cattle (186,596 local breeds and 9,182 exotic breed cattle), 9,033 sheep, 17,349 goats, 15,162 donkeys, 8,975 horses, 281 mules, 213,952 poultry (190,567 local breeds and 23,385 exotic breeds), and 9,566 beehives [30] (See Figure 1 below the map for the location of the study area).

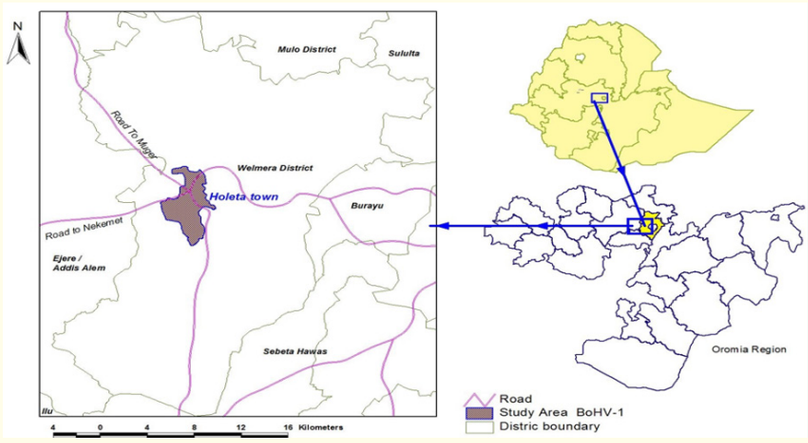

Figure 1: Map showing the study area [31].

Study animals

The study was conducted on dairy animals, particularly Holstein Frisian (HF) and cross-breed cattle. The study included animals of all age groups over six months and of both sexes.

Study design and sample size determination

A cross-sectional study was conducted in dairy farms of Holeta town from March 2021 to November 2021. Sample size was determined according to the Thrusfield formula [32] based on the 41%seroprevalence of the disease in Central Ethiopia reported recently by Sibhat [15], according to the equation 1 below.

$$n = \frac{1.96^2 * Pexp\ (1-Pexp)}{d^2} \quad \text{----(1)}$$

Where; n = required sample size

Pexp = expected prevalence

d = desired absolute precision

With 5% desired precision, a 95% confidence level is considered. Accordingly, the calculated sample size was 372. However, to increase the precision, the sample size was increased by 4.8% to 390.

Sampling techniques

Simple random sampling approaches were used to select 16 study herds from 36 registered dairy farms, and individual animals were sampled by cluster sampling techniques. Ultimately, 390 serum samples were obtained from 16 selected dairy herds. A total of 27, 106, and 257 sera samples were collected from small, medium, and large-scale dairy herds, respectively, with calves less than 6 months of age being excluded to avoid bias or false positives (i.e., serological responses induced by colostral antibodies).

Blood sample collection

About five to seven milliliters (5-7 ml) of blood samples were collected aseptically from the jugular vein using vacutainer tubes of 10mlvolume without anticoagulant and held at room temperature overnight to enable the separation of serum [15,26]. These sera were decanted into sterile cryovials of 1.8 ml volume, labeled, and shipped in a cold chain to Animal health institute (AHI) Sebeta, Ethiopia, where it was stored at -20°C until a laboratory test was performed.

Questionnaire survey

A close-ended questionnaire was designed to collect information on factors that influence the spread and prevalence of BoHV-1 infection. During the study period, general information on IBR was acquired from dairy farm owners or animal attendants on each farm via questionnaire interviews. The respondent's (i.e., farm owners, attendants, or any family members) participation in the study was voluntary, and no general information regarding the herd was withheld.

Diagnostic techniques

All sera samples were tested for the presence or absence of antibodies against BoHV-1 antigen immobilized on the microplate following the manufacturer's instructions for the indirect ELISA kit (IDEXX IBRgBX3-ELISA, Indirect, Switzerland). The bound antibody was detected using enzyme-labeled anti-bovine immunoglobulin antiserum. After 5x washing, the unbound conjugate is washed away, and with the addition of a substrate, the product reacts with the chromogen to generate a blue colour. Upon the addition of the stopping solution, the yellow colour was generated. The absorbance at a single wavelength of 450nm was measured by the spectrophotometer (EL*x*808, UK).Blocking% was calculated as ((Mean OD450 value of the negative control - OD450 value of the sample)/(Mean OD450 value of the negative control)) x100. The kit

was validated for testing BoHV-1 antibody in bovine serum samples and computed in order to interpret the results. The test was deemed to be valid if both the mean value of the negative control OD (OD_{NC}) is greater than or equal to 0.500 ($OD_{NC} \geq 0.500$) and the mean Blocking% of the positive control is greater than 80 (Mean PC Blocking% > 80). Any sample with a blocking%: <45%, 45% ≤ Blocking% < 55%, and ≥ 55%, was regarded as negative, doubtful, and positive, respectively.

Data management and analysis

The collected data were entered, cleaned, and coded into a Microsoft Excel Spreadsheet. The data was transported to Stata/SE13.0 [33] software for analysis. The prevalence of BoHV-1 infection was calculated as the number of IBRgB-ELISA positive sera samples divided by the total samples examined and multiplied by 100. The association of various risk factors was explored first using univariate logistic regression, and further multivariate logistic regression analysis was built to observe the presence of the association of potential risk factors (herd size, origin, age, sex, respiratory problems, parity, repeat breeding, and abortion) with the outcome variable of animals' seropositivity was determined. Statistical significance was set at 0.05 alpha value where $\rho < 0.05$ were considered significant.

Results

Serological results

In the current study, out of 16 dairy herds and 390 sera samples tested by IBRgB-ELISA, 93.75% at the herd level and 32.6% at the individual level were seropositive for BoHV-1 antibody detection, as shown in Table 1.

Different risk factors were considered in this study to determine the relationships with an outcome variable. Of which BCS ($\chi2 = 7.6835$, $\rho = 0.021$) showed significance with its categorical percentages of good (60%), lean (29.9%), and catchectic (33.3%). Animals with respiratory problems showed significantly higher seroprevalence (87%)($\chi2 = 32.9286$, $\rho = <0.001$) compared to those without respiratory problems (29.1%). Likewise, higher seroprevalence (50%) was also observed in repeat breeders ($\chi2 = 4.1761$, $\rho = 0.041$) compared to non-repeaters (31.2%). The remaining factors (herd size, origin, sex, age, abortion, and parity) did not show significant association with BoHV-1 infection in the current study, as indicated in tables 2, 3, and 4.

As illustrated in table 5 below, univariate logistic regression analysis showed that the likelihood of getting a seropositive animal was 16.2 times higher in animals with respiratory problems compared to those without respiratory problems ($\rho < 0.001$). Similarly, the risk/odds of being positive for *BoHV-1*was higher for good BCS (COR = 0.3; $\rho = 0.026$) than for catchectic (COR = 1.2; $\rho = 0.494$) and lean. There is a significant variation in repeated breeders' ($\rho = 0.020$) as well. However, the remaining explanatory variables did

Table 1: The overall BoHV-1 seropositivity.

District	No. of samples tested	No. of positive	% Positivity	95% CI
Holeta	390	127	32.6	28.1 - 37.4

Table 2: BoHV-1 seropositivity based on herd size and animals origin.

Variables	Categories	No of examined samples	Total no of positive (%)	χ^2	ρ–Value
Herd size	Small	27	7 (27.92%)	4.4255	0.109
	Medium	106	43 (40.57%)		
	Large	257	77 (29.96%)		
Origin	Purchased	21	7 (33.33%)	0.0060	0.938
	Borned	369	120 (32.52%)		

Table 3: BoHV-1 seropositivity based on sex, age, BCS, and respiratory problems.

Variables	Categories	Nº of examined samples	Total Nº of positive (%)	χ^2	ρ – Value
Age	6 month -1 year	103	30 (29.13%)	1.4624	0.481
	1-2years	52	15 (28.85%)		
	>2years	235	82 (34.89%)		
Sex	Male	39	12 (30.77%)	0.0636	0.801
	Female	351	115 (32.76%)		
BCS	Catchectic	129	43 (33.33%)	7.6835	0.021
	Lean	241	72 (29.87%)		
	Good	20	12 (60%)		
Respiratory problem	Present	23	20 (86.96%)	32.9286	< 0.001
	Absent	367	107 (29.15%)		

Table 4: BoHV-1 seropositivity based on parity, abortion history and repeated breeding.

Variables	Categories	Nº of examined samples	Total Nº of positive (%)	χ^2	ρ –Value
Parity	Primiparous	70	30 (42.86%)	5.2072	0.074
	Pluriparous	187	61 (32.62%)		
	Nulliparous	133	36 (27.067)		
Abortion	Aborted	28	5 (17.86%)	7.1847	0.028
	Non-aborted	229	86 (37.55%)		
	Neither	133	36 (27.07)		
Repeat breeder	Repeater	28	14 (50%)	5.8233	0.054
	Non-repeater	229	77 (33.62%)		
	Neither	133	36 (27.067)		

Table 5: Univariable and multivariable logistic regression results.

COR = Crude Odd Ratio, AOR = Adjusted Odd Ratio, * = Reference category.

Variables	Categories	Nº of examined samples	Nº of positive (%)	COR (95 CI)	ρ-Value	AOR (95%CI)	ρ-Value
Herd size	Small	27	7(27.9)	*	-	*	-
	Medium	106	43(40.6)	0.5(0.2-1.3)	0.166	0.4(0.1-1.3)	0.132
	Large	257	77(29.9)	0.8(0.3-2.0)	0.663	0.9(0.3-2.5)	0.817
Origin	Borned	369	120(32.5)	*	-	*	-
	Purchased	21	7(33.3)	1(0.4-2.6)	0.938		
Age	1-2 year	52	15(28.8)	*	-	*	-
	6 month - 1 year	103	30(29.1)	1(0.5-2.1)	0.971	*	-
	>2years	235	82(34.9)	0.8(0.5-1.3)	0.300	-	-
Sex	Male	39	12(30.77)	*	-	*	-
	Female	351	115(32.76)	0.9(0.4-1.9)	0.801	-	-
BCS	Lean	241	72(29.9)	*	-	*	-
	Catchectic	129	43(33.3)	1.2(0.7-1.9)	0.494	1.3(0.8-2.2)	0.271
	Good	20	12(60)	0.3(0.1-0.9)	0.026	0.3(0.1-1.0)	0.057
Respiratory problem	Absent	367	107(29.15)	*	-	*	-
	Present	23	20(86.95)	16.2(4.72-55.56)	< 0.001	18.79(5.23-67.55)	< 0.001
Parity	Nulliparous	133	36(27.1)	*	-	*	-
	Pluriparous	187	61(32.62)	0.77(0.4-1.25)	0.287	1.32(0.35-4.97)	0.682
	Primiparous	70	30(42.86)	0.5(0.27-0.91)	0.02	0.72(0.19-2.80)	0.641
Abortion	Aborted	28	5(17.86)	*	-	*	-
	Neither	133	36(27.1)	0.59(0.21-1.66)	0.313	1	-
	Non-aborted	229	86(37.55)	0.36(0.13-0.99)	0.047	0.3(0.1-0.9)	0.033
Repeated breeder	Neither	133	36(27.1)	*	-	*	-
	Non-repeater	229	77(33.6)	0.73(0.46-1.17)	0.195	2.30(0.99-5.36)	0.054
	Repeater	28	14(50)	0.37(0.16-0.85)	0.020	1	-

not show statistical association with determining the infection status in the current study, except for abortion and respiratory problems for multivariate logistic regression.

Results of questionnaire survey

Table 6: Results of a questionnaire survey on BoHV-1 in the study area.

Risk factors	Categories	Frequency	Percent (%)
Herd size	Small	3	18.75
	Medium	8	50
	Large	5	31.25
Service type	AI	7	43.75
	Bull	9	56.25
Time of abortion	< 4 month	3	18.75
	4-8 month	13	81.25
Abortion case history	Yes	16	100
Category of animals mostly aborted	Heifers	1	6.5
	Cows	12	75
	Equal	3	18.75
Forms of foetal disposal	Deep burial	5	31.25
	Left for carnivorous	11	68.75
Housing and ventilation	Poor	1	6.5
	Satisfactory	2	12.5
	Good	9	56.25
	Very good	4	25
Hygiene	Poor	1	6.25
	Satisfactory	1	6.25
	Good	10	62. 5
	Very good	4	25
Use of protective equipment (parturition)	Yes	7	43.75
	No	9	56.25
Culling criteria	Infertile, old aged and poor milk producer	8	50
	Old aged	4	25
	No	4	25

Discussion

In this study, out of 16 dairy herds and 390 sera samples tested, 15 (93.75%) at herd level and 127 (32.6%) at individual-level prevalence were recorded against *BoHV-1* infection, respectively. This indicates that BoHV-1 infection is well-established and widely distributed in improved dairy cattle in the study area. The result could be related to the fact that uncertified bulls are frequently utilized for production in the research area, as well as undiagnosed animals being purchased and combined with disease-free herds [24,34].

The seroprevalence of IBR found in this study was higher than the findings of 25.6% (85/332) recently reported by [16] in Dessie and Kombolcha towns in the aSouth Wollo Zone of the Amhara Region, North-Central Ethiopia. Similar studies in several countries indicated that the seroprevalence of IBR was higher than the present finding 36% in China [35], 43% in England [11], 60.84% in India [36], 63.54% in Southern India [37], 64.5% in Mexico [38], 83.33% in Western Kordofan [39], and 93.75% in Egypt from cattle imported from Sudan [40]. However, the current study's individual-animal level seroprevalence was comparable to the previous results [39], who found a total seroprevalence of 32% in Sudan's Nile River.

Similar studies conducted in different countries showed that the seroprevalence of IBR was lower than the present finding; 17.4% in Kenya [40], 19.5% in Turkey [41], 20.9% in Kenya [42] and 24.19% in Algeria [43]. In addition, the current finding was lower than the findings reported by [13-15] with a seroprevalence of 41.8% in Harar and Sidamo provinces, 67% in Gobe and Ghibe, and 41.0% in Central Ethiopia, respectively. Therefore, as there has never been a vaccine for IBR in Ethiopia, seropositivity to *BoHV-1* is a result of natural exposure to the virus [15,16]. This shows that, according to the findings of many scholars, the IBR seroprevalence was higher in various parts of the world, ranging from 17.4% to 93.75%. In contrast to Ethiopia, where there is no immunization, the antibodies observed in these nations could be attributable to immunization.

Seropositivity for BoHV-1 antibodies was 27.92%, 40.57%, and 29.96% in small, medium, and large herd sizes, respectively. According to the reports of Sibhat [15], small-holder farms have the lowest seroprevalence, followed by commercial and breeding farms. Due to the circulating virus in susceptible herds and *BoHV-1*'s infectious nature, the chance of seropositivity escalation gradually increases as the number of animals in a herd(s) grows, even if a single latently infected bovine exists within that herd(s). Based on age, older cows were relatively more prone to infection with *BoHV-1* (34.89%) than younger ones (29.13%), which was in agreement with the reports of Brock [44] in Irish cattle, where the total proportion of seropositive animals was low (20%) in younger animals (0-2years), and increased significantly in cattle aged 2-5years (20% to 70%). The possible justification for the increase in IBR prevalence with age might be that, as animals get older, they are more likely to come into contact with other animals that have recovered from the disease but are still carriers [6,44].

Based on the origin of the animals in the current study, 33.30% of the animals were purchased and 32.52%) were house-borne animals, which were found to be seropositive for *BoHV-1* antibodies. The result was reciprocally consistent with the reports of Dias [45] and Sibhat [15], where the seroprevalence of IBR was (36.70%) in purchased and (44.60%) in house-born animals. As stated previously by Van [46], this finding could be due to the fact that newly purchased and introduced animals might be the source of BoHV-1 viral infection to help spread the virus into disease-free herds.

Regarding the reproduction problems, seropositivity for BoHV-1 antibodies detections, 50% were repeaters and 33.6% were non-repeaters, which could be in agreement with the justification of Sibhat [15] where they described that reproductive disorders, which include abortion and repeated breeding, can affect both ovaries and corpus luteum where they are susceptible to BoHV-1 viral replication and pathogenesis [47,48]. These disorders could be the result of infection with virulent strains of BoHV-1.1 and BoHV-1.2a in cows [49]. In this research, the prevalence of IBR concerning reproductive disorders, particularly repeated breading, has revealed a clear link between seropositivity and the occurrence of these diseases ($\rho = 0.020$). As a result, animals with a history of reproductive issues, such as repeat breeding and abortion, have a higher prevalence of IBR. Repeat breeders were 0.37 times more likely to be at risk than non-repeat breeders in this study, while dairy cows that had not been aborted were 0.3 times more likely to be protective than dairy cows with a recorded abortion history. Furthermore, as compared to apparently healthy animals, animals with a history of reproductive issues had higher seroprevalence [15,16,37].

However, there was a statistically significant difference in IBR prevalence between the primiparous 42.86% and nulliparous 27.07% dairy cows, with primiparous having a risk of 0.5 times that of nulliparous. The current finding also contradicts with a recent study [16] which found that the risk of pluriparous (33.50%) and primiparous (14.90%) dairy cattle is 2 times higher in pluriparous than in primiparous. Similarly, in the Netherlands, just 49% of first calf cows were seropositive, compared to 91% of older cows [50].

In terms of BCS, seropositivity for BoHV 1 antibodies, catchectic 33.33%, lean 29.87%, and good 60% were all positive. Furthermore, IBR seroprevalence was somewhat greater in females (32.76%) than in males (30.77%). The current study's findings agreed with those of other studies [51], which found that BoHV-1 antibody detections were more common in females (12.35%) than in males (5.80%). Similarly, it was also reported that there was more IBR prevalence in females than in males [37]. The possible reason for higher seropositivity in females might be due to the use

of infected semen or seropositive bulls for insemination or breeding purposes [38].

Regarding the respiratory problem predictive variable, animals with respiratory problems showed 86.95% and without respiratory problems 29.15%, which is in line with the arguments of Fulton [28], where seropositive animals for BoHV-1 antibodies were associated with different clinical signs exhibited by the infected host. This would be due to BoHV-1 infections, most likely associated with IBR as stated by Graham [52], in which the clinical signs are inflammation of the nose (rhinitis) and trachea (tracheitis) that cause respiratory problems even though BVDV, BPI-3V, and BRVSV were associated with BRD [2,3]. The difference in seroprevalence across countries may be due to variations in sample size, sampling frame, study design, study periods, breeds of animals, geographical locations, management systems (such as housing, feeding, and hygiene), usage of uncertified bulls, and bull sharing between neighboring farms for natural services, semen from the infected bull for AI, and the specificity and sensitivity of the kits used for diagnostic purposes by these countries [6,24,34].

Clinical signs of IBR disease are not always observed in the dams, and abortion usually occurs in the third trimester of pregnancy. In this study, abortion history was registered from 17.86% for aborted and 37.55% for non-aborted animals that were seropositive for BoHV-1 antibody detection, respectively. This result showed that the factors could not exactly define the nature of the disease, which was in line with the reports of SAC. Bovine abortions were determined to be less than 2% due to BoHV-1 in Scotland between 2004 and 2008, according to an earlier survey in the UK as it was reported by SAC [53]. Abortions caused by natural BoHV-1 infections are most common, and animals are aborted between 4 and 8 months of pregnancy [49]. According to the questionnaire results, 81.25% of the dairy cows were aborted between 4and8 months of gestation. However, foetal mortality is considered to occur within 24–48 hours of foetal infection, but the fetus can be kept for up to 7 days before termination [15].

During the research period in the study area, 100% of voluntarily interviewed individuals responded to the presence of an aborted animal(s) in their herds in the past, with abortion case history recorded by at least one or more than one heifer and/or cow in each herd. Based on the respondents, 18.75% and 81.25% were aborted in less than 4 months and between 4-8 months, respectively. Furthermore, cows 75% were more likely to be aborted than heifers 6.25%. Regarding the usage of services for cows during the heat or estrus period, 34.75% of interviewees offered AI and 56.25% used a bull (natural) service. According to the researchers, the high seropositivity may be attributed to the use of uncertified bulls and bull sharing with neighboring dairy farms. This practice predisposes a disease-free herd to be affected by the virus by direct contact during mating and/or by virus-contaminated semen obtained from latently infected bulls [6]. Based on the diagnostic history for IBR since the establishment of their farms, no dairy farms were tested and no respondents knew about the disease, except for one respondent (who is a veterinarian).

Conclusion and Recommendation

The current study clearly showed that the high seropositivity of dairy cows (32.6%) for BoHV-1 infections in the study area was indicative that the wild strain (field strain) of BoHV-1 is commonly circulating on almost all dairy farms. And this finding indicates that the disease is easily spread and distributed within the herd and, most likely, to neighboring farms. Based on a multivariate logistic regression model, those dairy cows having respiratory problems were more at risk than those that didn't have respiratory problems. Hence, risk factors such as respiratory problems, parity, and repeated breeding were significantly associated with BoHV-1 infection in this study area. Based on the current finding of serological detection of BoHV-1 and its identified risk factor, the following recommendations were forwarded: (1) it is suggested that a study of BoHV-1 infection in an experimental animal be conducted for additional virus isolation, viral DNA sequencing, and sub-typing. (2) Owners of dairy farms need to be made more aware of the disease's economic effects, risk factors that are linked to it, methods for prevention and control, and mode of transmission. (3) Finally, although it is not recommended to cull those seropositive animals, given Ethiopia's current economic situation, isolation from the herd should be necessary.

Ethical Statement

This research was allowed and approved to be conducted by the Animal Research Scientific and Ethics Review Committee of the Animal Health Institute, Sebeta, Ethiopia, with a Certificate Ref. No. of ARSERC/EC/019/04/06/2021.

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Author Contributions

CD: conceived the research idea, prepared the research proposal, collection of data, and performed an indirect-ELISA test, manuscript drafting and original research writing. KA: participated in proposal preparation, supervising, commenting, and manuscript reviewing. DZ: participated in proposal preparation, data analysis and interpretation, and reviewed the manuscript. The final manuscript was reviewed and approved by all authors.

Disclosure

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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