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Seroprevalence of bovine Herpesvirus-1, bovine viral diarrhoea virus, *Neospora caninum* and *Coxiella burnetii* in dairy cows in Ethiopia

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Abstract

Background The reproductive problem is an animal health-related bottleneck that constrains livestock genetic improvement efforts in tropical countries such as Ethiopia. The infectious causes of reproductive disorders are one cause of decreased reproductive efficiency. This study aimed to determine the seroprevalence to Bovine Herpesvirus-1 (BHV1), Bovine Viral Diarrhea Virus **(**BVDV), *Neospora caninum* (*N. caninum)* and *C. burnetii (C. burnetii)* exposures in dairy cows with reproductive disorders in selected areas of Ethiopia. Overall, 164 serum samples were collected from October 2018 to May 2019 from animals with a history of reproductive disorders. The collected sera were tested for antibody titers to *Brucella* species, *N. caninum*, BVDV, BHV1, *C. burnetii* and *Chlamydophila abortus* (C. *abortus*) using Rose Bengal and ELISA.

Results The apparent seroprevalence of BHV1, BVDV, *N. caninum* and *C. burnetii* were 61%, 33.5%, 4.9% and 0.6%, respectively. Among the selected study areas, the mean apparent seroprevalence was significantly greater in Bishoftu (35.9%), Holeta (34.2%) and Adaberga (28.6%) than in Mekelle (9.9%) and Ambo (16.2%). Among the specific seroprevalence in specific areas, BHV1 was the most common in Adaberga, with an apparent seroprevalence of 92.9%. Similarly, the seroprevalence of BVDV was the highest in Holeta, with an apparent seroprevalence of 73.3%. On the other hand, no seropositive animal to *Brucella* spp. or *C. abortus* was found in these study areas.

Conclusion BVDV and BHV1 seroprevalence was higher in dairy cattle with a history of reproductive disorder in Ethiopia as compared to the seroprevalence of *N. caninum* and *C. burnetii*.

Keywords Reproductive problems, Dairy cattle, Ethiopia

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Introduction

Reproductive performance is one of the desired production traits in dairy farming [1]. The performance of dairy cattle mainly depends on their genetic makeup, health, nutrition, husbandry and environment. In sub-Saharan Africa, efforts to improve livestock productivity through cross-breeding of exotic breeds with local animals have been partly limited by reproductive problems. In addition to the noninfectious causes of reproductive problems such as heat stress, toxins and poor nutrition, reproductive disorders caused by bacteria, viruses, protozoa, and fungi play an important role in the development of reproductive problems in animals [2–5]. Reproductive failure of dairy cattle, due to its detrimental effects on overall fertility, delayed calving intervals, medication costs, decreased milk production and decreased production of potentially productive cattle, results in significant economic losses in the dairy industry [6]. Culling due to reproductive failure is also a source of huge economic loss [7]. Some reports indicate that, globally, almost half of abortion and stillbirth cases are caused by infectious agents [8].

Globally, infectious causes of reproductive problems have become a serious challenge to the dairy industry. The most commonly involved bacterial pathogenic agents are *Brucella abortus*, *C. abortus*, *C. burnetii* [9–12]. In addition to bacterial pathogens, viruses such as bovine viral diarrhea virus (BVDV), Bovine Herpesvirus-1 (BHV1), and parasites, including *N. caninum* and *Trichomonas fetus*, are commonly associated with reproductive problems [13]. Such infectious causes of reproductive constraints reduce the production and productivity potential of dairy animals and limit livestock and livestock products to market access, and some pathogens can even pose a significant health risk to the public [14].

Although different terms and syndromes, such as dystocia, retained fetal membrane (RFM), metritis, embryonic death, abortion, infertility and repeat breeding are used to explain reproductive problems in dairy cattle, abortion and infertility are the most significant problems affecting dairy herds. Due to the complex nature of the pathogenic agents, the specific cause of abortion and/or infertility in cattle cannot be easily determined by observing simple clinical signs or syndromes [15].

Moreover, the underlying causes and distribution of reproductive problems in dairy cattle have been studied. In Ethiopia, there is certain information on the types of dairy cattle reproductive problems and their extent. However, most of these studies focused only on a single exposure to pathogenic agents like the prevalence of BVDV (32.6%, 450/1379), *N. caninum* (13.3%, 310/2334), BHV1 (41.0%, 565/1379), bovine *Brucella* spp. (1.3%, 13/967) and *Toxoplasma gondii* (10.74%, 35/326) as reported by the authors [16–20], respectively. Fragmented studies such as this one may not have enough evidence at the same time to determine the exact problem. To improve the productivity of dairy cattle, understanding overall husbandry and animal health-related constraints such as reproductive problems under different production conditions is important. Exploring further evidence on the reproductive problems of dairy cattle in Ethiopia will help to design specific and effective disease prevention and control strategies that are customized to the existing situation. Therefore, the present study aimed to determine the seroprevalence to four reproductive diseases in dairy cows with a history of reproductive disorders in selected areas of Ethiopia.

Main text

Methods

Study area

The study was carried out from October 2018 to May 2019 in different agro-ecological areas of Ethiopia where higher milk production occurs. Most of the study areas were from two zones (East and West Shewa) of the Oromia region and Mekelle, the capital city of the Tigray regional state (Fig. 1). Bishoftu from East and Holeta, Adaberga and Ambo from West Shewa were selected based on their contributions to the milk shade potential of Addis Ababa, the capital city of Ethiopia. Agro-ecological classification is mainly based on altitudinal variations that have a strong impact on temperature and rainfall and consequently on agricultural land uses, mainly crop and animal production. The highlands, midlands, and lowlands cover altitudinal ranges of 2300–3200, 1500–2300, and 500–1500 m above sea level, respectively. Accordingly, the study was conducted in middle land areas (Bishoftu, Ambo and Mekelle) and highland areas (Holleta and Adaberga).

Bishoftu town is located 45 km southeast of the capital city, Addis Ababa. The area is located at 9°N latitude and 40°E longitude at an altitude of 1850 masl, with an annual rainfall of 866 mm, 84% of which occurs during the long rainy season from June to September (NMSA, 2010). Ambo town is the administrative center of the West Shewa zone and Ambo district. It is located at a latitude and longitude of 8°59 N 37°51′E to 8.983°N 37.85°E and an elevation of 2101 meters above sea level and is located 114 km west of Addis Ababa. It has annual rainfall and temperatures ranging from 800–1000 mm and 20–29°C, respectively. Mekelle is the capital city of the Tigray regional state, northern Ethiopia. Geographically, it is located between 13° 23' north latitude and 39° 29' east longitudes at altitudes of 2070 m above sea level. The mean annual rainfall ranges from 250 to 300 mm, and the temperature ranges from 12 °C to 27 °C.

Holeta is situated 33 km west of Addis Ababa. The dairy farms lie at approximately 38° 30' E longitude and

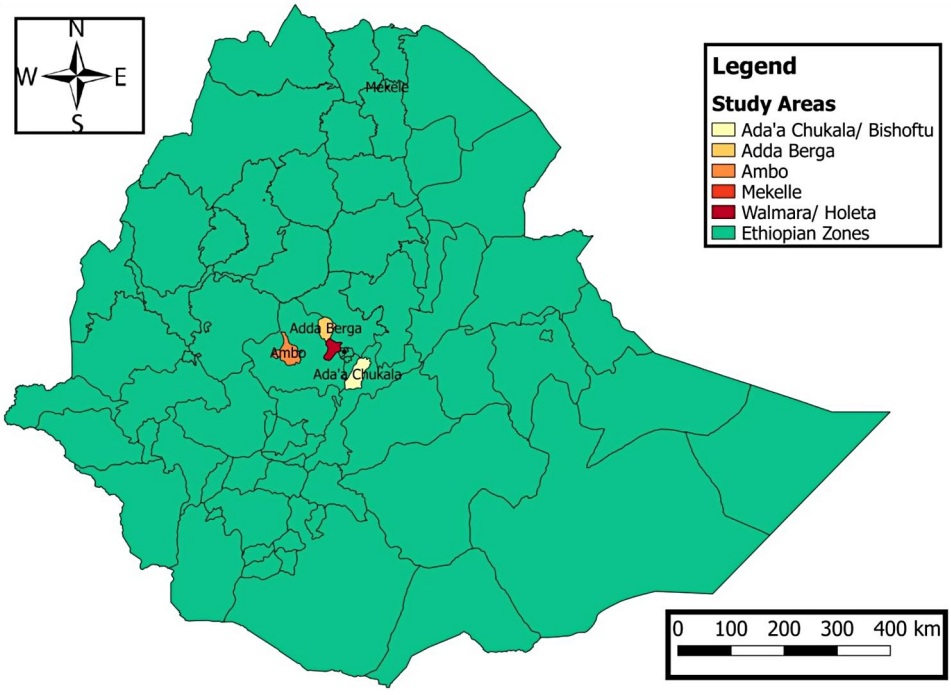

Fig. 1 Study areas

9° 3' N latitude and 2400 m above sea level. The area is characterized by a moderately cold climate with a temperature of 6–22 °C. It has a mean relative humidity of 59%. The annual rainfall ranges from 818 to 1247 mm. Adaberga is located at 90 16'N latitude and 380 23'E longitude. The annual temperature and rainfall range from 18 °C to 24 °C and from 1000 to 1225 mm, respectively. Both Holeta and Adaberga have bimodal rainfall patterns with short rainy periods from March to May and long rainy seasons from June to September. Both dairy farms have a semi-intensive farming system where the animals frequently spend their time grazing and indoor feeding (Holeta Agricultural Research Center, 2008).

Study design and sampling

All dairy cattle found in the study dairy farms were taken as a target population. All animals selected for the study were maintained under the same (semi-intensive) management system. Except two dairy farms owned by research centers (from Holeta and Adabarga), all dairy farms were owned by private farmers. A list of dairy farms with at least five years after establishment, and their number of dairy cattle were obtained from their agricultural offices in each of the study areas. As a result, three (3/27), four (4/42) and five (5/47) dairy farms were selected from Ambo, Bishoftu and Mekelle, respectively. Animals with a history of at least one reproductive disorder such as abortion, stillbirth, repeated breeding, dystocia, retained placenta, anoestrus, uterine prolapse, vaginal prolapse, prolonged uterine discharge and others was considered to meet the inclusion criteria. On dairy farms that had eight or less cows with reproductive disorders, all of the cows that presented with a history of reproductive disorders were sampled. Whereas, in dairy farms that had more than 8 animals with reproductive disorders, eight animals plus one third of the remaining cows with reproductive disorders were sampled. Simple random sampling was used to select the animals from the list that have a history of reproductive disorder.

Blood samples (10 ml) were collected from cattle ($n=164$) with a history of reproductive problems using sterile needles and plain vacutainer tubes from the jugular vein. The collected sera were decanted and transported to Holeta National Agricultural Biotechnology in ice packs and stored at -20 °C until screening. Sample collection and serological analysis were performed as per the recommendation of [21]. For the purpose of this study, loss of the fetus between 42 and 260 days of gestation was considered abortion, and a calf born dead between 260 days and full term or who died within 24 h following birth was considered stillborn.

Laboratory analysis

The collected serum was tested for positive antibody titers to *Brucella* species, *Neospora caninum*, BVDV, BHV1 and *C. burnetii* using commercial serological kits. Serological analysis was conducted at Holeta National Agricultural Biotechnology Research Center Laboratory. Serum samples were screened using the Rose-Bengal Plate Agglutination Test to screen for antibodies against

Brucella species. Serum (30 µl) was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately equal to 2 cm in diameter. The mixture was rocked gently for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was graded as positive and otherwise negative, as indicated by [22]. After the *Brucella* antibody was screened using the rose bengal test (ID.vet, France), the *Brucella* spp. serum indirect multispecies test ELISA kit was used. The interpretation was based on the S/P% value, where <110% was considered negative, 110–120% was considered inconclusive, and >120% was considered positive. The detection of BHV1 was performed indirectly by the BHV1 (ID.vet, France) kit. The interpretation of BHV1 exposure was calculated considering an absorbance cutoff value of <50% as negative and >60% as positive. BVDV exposure status was determined based on a competitive ELISA using the BVDV p80 Antibody test (ID.vet, France). Samples whose percentage of S/*N*<50 were considered as negative, while those whose percentage of S/*N*>50% were considered positive. The presence of antibodies to *N. caninum* was determined using an *N. caninum* indirect multispecies test kit (ID.vet, France). A serum sample with an absorbance value (S/P) of <40 was considered negative, 40–50 was considered inconclusive, and >50% was considered Neospora positive. The seropositivity of *C. burnetii* was determined by a monoscreen AbELISA (Bio-X Diagnostics, Belgium). A coefficient less than 37% was considered negative, and those greater than or equal to 37% were considered positive. The test protocol and interpretation of all ELISA tests were performed according to the manufacturer's instructions. The test was repeated for inconclusive results.

Data analysis

The data collected from laboratory investigations were entered into a Microsoft (Ms.) Excel spreadsheet for coding, cleaning and validation. The collected data were analyzed using StataSE15. Descriptive statistics were used to compute frequency and animal-level prevalence. To compare the seroprevalence to the four reproductive diseases in dairy cows with a history of reproductive disorders across locations, 95% confidence with 5% precision were used. Associations between seroprevalence to the four reproductive diseases in dairy cows with a history of reproductive disorders and different risk factors were assessed using a logistic regression model. The strength of the association was determined by the odds ratio. Statistical significance was determined at $p<0.05$.

Results

Apparent seroprevalence to four reproductive diseases in dairy cows with a history of reproductive disorders

The apparent seroprevalence was defined as the number of animals that tested seropositive by a diagnostic test divided by the total number of animals in the sample tested. The true seroprevalence is the actual number of seropositive animals divided by the number of individuals tested in the population. Before we started the analysis, diseases for which at least one positive sample was not available were not included in the model. Accordingly, seroprevalence to bovine *Brucella* spps. and *C. abortus* were excluded from the analysis because there was no seropositive results. As a result, the analysis was carried out for seroprevalence to the four important diseases (BHV1, BVDV, *N. caninum* and *C. burnetti*). The mean apparent seroprevalence to the BHV1, BVDV, *N. caninum* and *C. burnetti* was 25.0%. Seroprevalence to BHV1 was found to be the most prevalent exposure in the study areas, with a seroprevalence of 61%, followed by BVDV, with a seroprevalence of 33.5%, with statistically significant differences ($p<001$). The odds of a cow being seropositive for BHV1was 30 times greater than the odds of being seropositive for *Neospora.* The odds of BHV1 seroprevalence was 3 times greater than the odds of seroprevalence to BVDV, and the difference was statistically significant (Table 1).

The effect of location on the apparent seroprevalence in dairy cows with history of reproductive disorders

The mean apparent seroprevalence to the BHV1, BVDV, *N. caninum* and *C. burnetti* in the study areas was 25.0%. Generally, the model showed a statistically significant difference ($X^2=29.87$, $p<0.05$) in at least one of the different study areas. The apparent seroprevalence in Bishoftu, Holeta and Adaberga were significantly greater than those in Mekelle and Ambo. The odds of a cow being exposed to BHV1, BVDV, *N. caninum* and *C. burnetti* in Bishoftu was approximately five times greater than the cow in Mekelle. However, the odds of a cow

Table 1 Seroprevalence of BHV1, BVDV, *N. Caninum* and *C. Burnetii* exposure in dairy cows with reproductive disorder in Ethiopia

Disease causes	Animal examined	Number of Positives	Apparent seroprevalence (%)	95% CI	OR	X^2	*P* value
C. burnetii	164	1	0.6 [A]	0.09–4.2	0.1	233.0	< 0.001
BVDV	164	55	33.5 [B]	10.2–69.2	9.8		
BHV1	164	100	61.0 [C]	26.1–87.4	30.5		
N. caninum	164	8	4.9 [A]	2.5–9.4	1		
Total	**656**	**164**	**25.0**	**21.8–28.5**			

OR- odds ratio; CI- confidence interval, *Apparent prevalence with similar letters is not significantly different

being exposed to those pathogenic agents in Holeta was approximately four times greater than that in Mekelle. On the other hand, the odds of a cow exposed to the four pathogenic agents in Bishoftu, Holeta and Adaberga did not significantly differ. Similarly, no statistically significant difference was observed between the Mekelle and Ambo study areas (Table 2).

The effect of location on the apparent seroprevalence to each of BHV1, BVDV, *N. Caninum* and *C. Burnetti* in dairy cows with history reproductive disorders

Analyzing the apparent seroprevalence in individual reproductive diseases across locations is crucial for identifying specific control methods. In almost all (except one) of the study areas, seroprevalence of BHV1 was the most prevalent. The highest apparent seroprevalence to BHV1 was found in Adaberga (92.9%), followed by Bishoftu (69.6%). Side by side, the highest apparent seroprevalence to BVDV was found in Holeta (73.3%), followed by Bishoftu (69.6%). Despite its higher apparent seroprevalence in other areas, BVDV was not found at the Ambo site. Generally, the apparent seroprevalence of *N. caninum* and *C. burnetii* was found to be low or nonexistent in almost all the study areas. The apparent seroprevalence of *N. caninum* in Ambo was relatively greater (17.6%) than the results in the other study areas. There were almost no seropositive to *C. burnetii* in the study areas except for one (1/56) in Adaberga (1.8%), as indicated in Table 3. In addition, the seroprevalence to at least one of the four pathogenic agents across farms showed statistically significant difference ($X^2=38.2$, $p<0.001$). The odds of an animal being seropositive to at least one of the pathogenic agents in farms 7 & 8 is 4.9 and 4.7 times greater than the odds of an animal being seropositive to at least to one of the four pathogenic agents in farm 14 (Table 4). Furthermore, the seroprevalence to each pathogenic agent in each farm is indicated in Tables 5, 6, 7.

In 21% of the study farms (3/14), there was no an apparent evidence of exposure to *C. burnetii*, BVDV, BHV1 or *N. caninum* in cows with a history of reproductive problems. Statistically significant difference of seroprevalence was observed among the dairy farms ($X^2=38.2$, $p<0.001$) (Table 4). The odds of an animal to be seropositive to at least one of the four pathogenic agents in farm 7 was about 5 times higher than the odds of an animal to be seropositive to the four pathogenic agents in farm 14 (Table 4). On the other hand, more than 71% (10/14) and 57% (8/14) of the dairy farms were seropositive to BHV1 and BVDV, respectively (Fig. 2). A farm is said to be a seropositive when at least one animal from the farm is found to be seropositive to the pathogenic agent.

Further seroprevalence analysis at each pathogenic agents in the fourteen dairy farms showed statistically significant differences (Tables 5, 6 and 7). 100% seropositivity to BHV1 was found in farms 7 and 11 (Table 5). Similarly, 100% seropositivity to BVDV was found in farms 3, 4, 5 & 8 (Table 6). On the contrary, higher seropositivity to *N. caninum* (75%) was found only in one farm (Farm 8). As there was only one seropositive animal to *C. burnetii* from the 14 dairy farms, no statistical analysis was carried out for *C. burnetii*.

Discussion

This study reported the seroprevalence of four pathogens in dairy cattle kept under semi-intensive production systems in the selected areas of Ethiopia. Dairy farms follow semi-intensive managements where animals depend on open grazing for green fodder and on barn concentrate supplementation and water provision. The mean apparent seroprevalence to the four pathogenic agents in the dairy cattle with reproductive disorder was about 25.0%. Apparent seroprevalence to infectious bovine rhinotracheitis was found to be the most prevalent in the study areas, with an apparent seroprevalence of 61%, followed by the seroprevalence to the bovine viral diarrhea virus, with an apparent seroprevalence of 33.5%. These results were similar to previous findings in dairy cattle which had reproductive disorders in Sudan, which indicated that reproductive problems in dairy cattle are the main bottlenecks of smallholder dairy production [23]. Relatively higher seroprevalence to IBRV (74%, 17/230) and BVDV (58%, 141/243) from dairy cattle with reproductive disorder was found in brazil [24].

Table 2 The mean seroprevalence (BHV1, BVDV, *N. Caninum* and *C. Burnetii* exposure) in dairy cows with reproductive disorder in different regions in Ethiopia

Study areas	Animal examined	Number of Positives	Apparent seroprevalence (%)	95% CI	OR	X^2	*P* value
Mekelle	152	15	9.9 [A]	4.3–21.2	1	37.42	< 0.001
Adaberga	224	64	28.6 [B]	23.0-34.8	3.6		
Bishoftu	92	33	35.9 [B]	20.0-55.6	5.1		
Ambo	68	11	16.2 [A]	6.6–34.4	1.8		
Holeta	120	41	34.2 [B]	19.4–52.7	4.7		
Total	**656**	**164**	**25.0**	**21.8–28.5**			

OR- odds ratio; CI- confidence interval

*Apparent seroprevalence with similar letters is not significantly different

Table 3 The effect of locations on the apparent seroprevalence of BHV1, BVDV, *N. Caninum* and *C. Burnetii* exposure in dairy cows with reproductive disorders

Study areas	Disease types	Animal examined	Number of Positives	A. seroprevalence (%)	95% CI	OR	X^2	*P* value
Bishoftu	BHV1	23	16	69.6	21.1–95.1	1	30.76	< 0.01
	BVDV	23	16	69.6	21.1–95.1	1		
	N. caninum	23	1	4.3	0.2–49.7	0.02		
	C. burnetii	23	0	0	-			
	Total	**92**	**33**	**35.9**	**26.8–46.1**			
Holeta	BHV1	30	16	53.3	14.7–88.3	1	54.40	< 0.01
	BVDV	30	22	73.3	55.0-86.1	2.4		
	N. caninum	30	2	6.7	0.6–45.4	0.06		
	C. burnetii	30	1	3.3	0.2–40.0	0.03		
	Total	**120**	**41**	**34.2**				
A.berga	BHV1	56	52	92.9	66.7–98.8	53.2	128.9	< 0.01
	BVDV	56	11	19.6	12.2–32.1	1		
	N. caninum	56	1	1.8	0.1–22.0	0.07		
	C. burnetii	56	0	0	-	-		
	Total	**224**	**64**	**28.6**	**23.0-34.8**			
Ambo	BHV1	17	8	47.1	5.1–93.7	4.1	3.45	0.063
	BVDV	17	0	0	-	-		
	N. caninum	17	3	17.6	5.8–42.7	1		
	C. burnetii	17	0	0	-			
	Total	**68**	**11**	**16.2**	**9.2–26.9**			
Mekelle	BHV1	38	8	21.1	3.3–67.3	1.4	7.27	0.026
	BVDV	38	6	15.9	7.3–31.0	1		
	N. caninum	38	1	2.6	0.1–36.1	0.14		
	C. burnetii	38	0	0	-	-		
	Total	**152**	**15**	**9.9**	**6.0-15.7**			

* A. seroprevalence - apparent seroprevalence, CI- confidence interval, OR- odds ratio

NB. While analyzing, the model excludes those that did not have at least one seropositive sample. As a result, the chi-square and p values are calculated from the samples that have at least one antibody positive sample

Table 4 Mean apparent seroprevalence for at least one of BHV1, BVDV, *N. Caninum* and *C. Burnetii* across different farms in dairy cows with reproductive disorders

Study Farms	Animal examined	Number of Positives	Apparent seroprevalence (%)	95% CI	OR	X^2	*P* value
1	224	64	28.57	7.8–65.4	2.4	38.2	< 0.001
2	12	0	0.00	-	-		
3	36	16	44.44	12.3–82.1	4.8		
4	12	4	33.33	5.4–81.3	3.0		
5	12	3	25.00	3.4–76.1	2.0		
6	40	1	2.50	0.1–31.1	0.2		
7	20	9	45.00	10.7–84.6	4.9		
8	16	7	43.75	9.6–85.0	4.7		
9	120	41	34.17	9.6–71.7	3.1		
10	24	4	16.67	2.5–61.1	1.2		
11	20	7	35.00	7.2–78.8	3.2		
12	28	0	0.00	-	-		
13	36	0	0.00	-	-		
14	56	8	14.29	7.3–26.1	Ref.		
Total	**656**	**164**	**25.00**	**21.8–28.5**			

In some studies, the apparent seroprevalence of BHV1 can vary widely. Hovingh and his colleagues reported that the seroprevalence of BHV1 ranged from 19.5 to 86% [25].This finding is more or less similar to our findings, with the lowest and highest ranges of 21.1% and 92.9% in the Mekelle and Adaberga groups, respectively. The higher seroprevalence of BHV1 might be attributed to the use of contaminated semen, stressful husbandry

Table 5 Apparent seroprevalence of bovine viral diarrhoea virus across different farms

Study farms	Animal examined	Number of Positives	Apparent prevalence (%)	95% CI	OR	X^2	*P* value
1	56	11	19.6	0.3–11.6	Ref.	37.7	< 0.001
2	3	0	0.0	-	-		
3	9	7	77.8	24.8–97.4	14.3		
4	3	1	33.3	2.1–92.1	2.0		
5	3	0	0.0	-	-		
6	10	0	0.0	-	-		
7	5	5	100.0	-	-		
8	4	0	0.0	-	-		
9	30	22	73.3	33.4–93.8	11.3		
10	6	3	50.0	8.4–91.6	4.1		
11	5	5	100.0	-			
12	7	0	0.0	-			
13	9	0	0.0	-			
14	14	1	7.1	0.5–55.8	0.3		
Total	**164**	**55**	**33.5**	**26.7–41.1**			

Table 6 Apparent seroprevalence of bovine Herpesvirus-1 across different farms

Study farms	Animal examined	Number of Positives	Apparent prevalence (%)	95% CI	OR	X^2	*P* value
1	56	52	92.9	82.5–97.3	117	43.7	< 0.001
2	3	0	0.0	-	-		
3	9	9	100.0	-	-		
4	3	3	100.0	-	-		
5	3	3	100.0	-	-		
6	10	1	10.0	0.4–75.4	Ref.		
7	5	4	80.0	11.4–99.2	36		
8	4	4	100.0	-	-		
9	30	16	53.3	10.6–91.6	10.3		
10	6	0	0.0	-	-		
11	5	2	40.0	3.0-93.5	6		
12	7	0	0.0	-	-		
13	9	0	0.0	-	-		
14	14	6	42.9	5.9–90.0	6.8		
Total	**164**	**100**	**61.0**	**53.3–68.1**			

Table 7 Apparent seroprevalence of Neospora caninum across different farms

Study farms	Animal examined	Number of Positives	Apparent prevalence (%)	95% CI	OR	X^2	*P* value
1	56	1	1.8	0.3–11.6	Ref.	15.5	0.004
2	3	0	0.0	-	-		
3	9	0	0.0	-	-		
4	3	0	0.0	-	-		
5	3	0	0.0	-	-		
6	10	0	0.0	-	-		
7	5	0	0.0	-	-		
8	4	3	75.0	2.0-99.8	165		
9	30	2	6.7	0.1–85.9	3.9		
10	6	1	16.7	0.1–96.4	11.0		
11	5	0	0.0	-	-		
12	7	0	0.0	-	-		
13	9	0	0.0	-	-		
14	14	1	7.1	0.1–90.5	4.2		
Total	**164**	**8**	**4.9**	**2.5–9.4**			

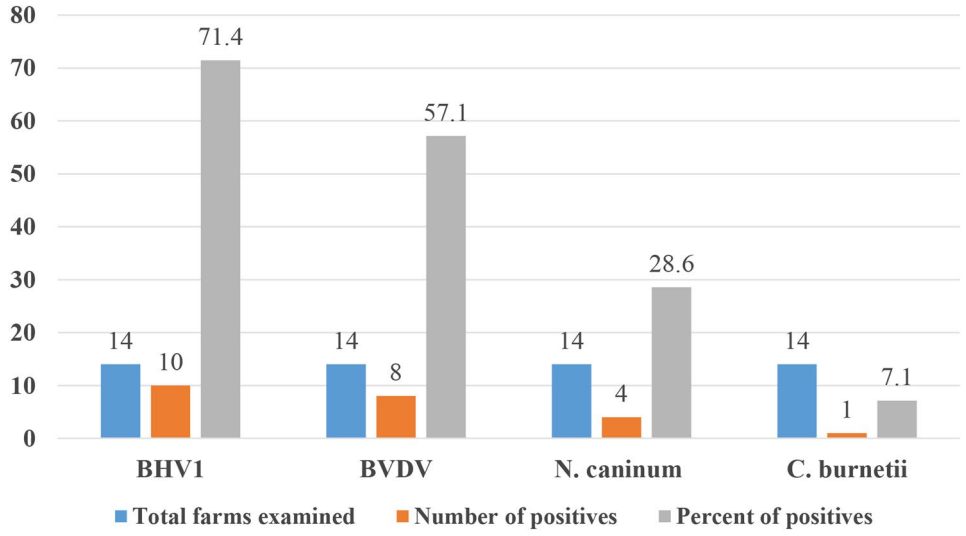

Fig. 2 Seropositivity of BHV1, BVDV, *N. caninum* and *C. burnetii* in forty dairy farms with a history of reproductive disorder

practices and frequent corticosteroid treatments, and the introduction of unscreened new cows/bulls to the herds. Similar to the BHV1, the seroprevalence of BVDV ranged from 0 to 73.3% in Ambo and Holeta, respectively. BHV1 and BVDV accounted for 94.5% (155/164) of seropositive results, and *N. caninum* and *C. burnetii* accounted for 5.5% (9/164) of seropositive results. The higher seroprevalence of BHV1 and BVDV over the *N. caninum* could be related to the pathogen transmission properties in that, BHV1 and BVDV are contagious pathogens whereas, *N. caninum* is a multi-host protozoal pathogen that needs mainly dogs as definitive hosts. Additionally, the birth of persistently infected calves by BVDV and once infected animals by BHVI remains infected throughout life. These infected cows can expose many other animals to the viruses by close contact at the time of viral shedding [24]. Whereas, the common route of *N. caninum* transmission is vertical transmission that is infection is from dam to calf in utero, which is why *N. caninum* is mostly maintained in family lines [26].

In the five study areas, more than 45% of the apparent seroprevalence was found in Bishoftu and Holeta. These areas are found in the Addis Ababa milk shade areas where a relatively higher density of dairy farms are found. The intensive management of dairy cows favors viral spread and increases the chances that healthy animals can come into contact with infected animals [27]. Relatively higher seroprevalence to BHV1 (74.7%, 133/178) and BVDV (49.2%, 58/118,) was found in South Africa from randomly selected dairy cattle [15]. On the other hand, previous results from Sudan indicated that reproductive problems in dairy cattle are the main bottlenecks of smallholder dairy production [23]. The semi-intensive dairy farming system in Ethiopia is vulnerable to viral disease transmission unless appropriate means of control are available.

Due to its improved specificity and sensitivity, an ELISA test [28] was used to detect seroprevalence to different pathogens from dairy cows in the present study. Since there is no history of vaccination for these pathogens in the study area, the detection of antibodies from tested cows implies that the animals were exposed to BHV1 and BVDV at some point in time [29].

In addition to BVDV and BHV1, *N caninum*, which is widely recognized as a cause of abortions in dairy cattle, was also diagnosed in the present study with a seroprevalence of 4.9%. A previous study in central and southern Ethiopia revealed a strong association between N. *caninum* sero-positivity and abortion in cattle [16]. A similar finding was also reported in New Zealand [30]; although comprehensive evidence on the disease burden of *N. caninum* in Ethiopian dairy cattle is lacking, the current study showed that *N. caninum* should not be ignored.

In the present study, the seroprevalence of *C. burnetii* was 0.6%. The observed prevalence was lower than that reported in Algeria, with an seroprevalence of 1.7% (6/354) from dairy cattle which had a history of abortion [31]. Similarly, a higher seroprevalence of *C. burnetii* (12.3%, 161/1,306) from bulk-tank milk of dairy cows from intensive farming was reported in Spain [32]. This could be related to the difference in farming system and presence of other animal species in close contact or vicinity to the dairy cattle farming. The presence of one or two infected animals in intensive farming can spread the bacteria to other animals by aerosol more easily than in semi-intensive dairy farms [33]. Sharing grazing areas of dairy cattle with sheep and goats can also increase the transmission of *C. burnetii* as the bacteria is transmitted in small ruminants via vaginal mucosa and faeces, while

transmission of the bacteria in dairy cattle can also occur via milk [34].

In spite of the fact that this finding reported higher overall seroprevalence to BHV1 and BVDV, about 21% (3/14) of farms were found to be seronegative of all the four pathogens from 76 animals with the history of reproductive disorders. This suggests that seropositivity may not correlate strongly with the presence of reproductive disorders and that there are other causes of reproductive failure beyond the infectious causes investigated in this study [35]. In support of this result, seronegative to BHV1 (0/24) was reported in Indonesia from cows with the history of abortion [36]. The seroprevalence of brucellosis in the current study was zero. Similar result was reported in Addis Ababa, Ethiopia, in studies of crossbred dairy animals using CFT [37].

Conclusion

According to this result, we can conclude that relatively higher seroprevalence to BVDV and BHV1 than *N. caninum* and *C. burnetti* was found in the dairy cattle with a history of reproductive disorders. The overall seroprevalence to these pathogens also varies from one study area to the other. Relatively higher seroprevalence to these pathogens were found in places where the Addis Ababa milk shade is found.

Limitations

The current study was conducted in dairy cattle with a history of reproductive disorders. In spite of the fact that the main purpose of this article was to compare and contrast the seroprevalence to the pathogenic agents attributing reproductive disorders in dairy cattle, it would be very nice had it include dairy cattle without a history reproductive disorder. A lack of disease recordkeeping on dairy farms is also common in Ethiopia. This also limits the number of sample size that would get from dairy farms. So, as a future research, detail investigation on these pathogens is needed from dairy herds with an appropriate record of reproductive diseases (abortion, stillbirth, failed to conceive, repeated breeding and others) with time of their occurrence; and samples need to be taken from animals with reproductive disorders and from animals without reproductive disorders using a case-control study design. Furthermore, the circulating pathogens need to be confirmed using molecular diagnostic techniques or viral isolation using cell culture.

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Author contributions
The research idea and study design were developed by YEM and GMW. Sample collection was performed by YEM, GG, BAE, SKB, WFG, BSD, DTT, STY, and BUH. YEM supervised the study. YEM and GG provided valuable information on the data analysis and manuscript writing. All the authors have read and approved the final manuscript.

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Data availability
The datasets used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval to conduct the study was obtained from the Ethiopian Institute of Agricultural Research. The Ethiopian Institute of Agricultural Research, established as federal agricultural research by Order No. 42 of 1966 and amended by Proclamation No. 79/1997 of the Federal Democratic Republic of Ethiopia, mandates research on animals, plants, and natural resources in the country. Before starting blood collection, letter of consent was read to district and areas leaders as well as farm owners stating the objectives of the research, and the right to refuse to participate in the research. Blood samples were then collected after verbal consent was obtained from these district and area leaders as well as farm owners. All research activities were conducted according to the rules and guidelines of the research institute.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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