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Serological evidence of Bovine herpesvirus-1, Bovine Viral Diarrhea virus and Schmallenberg v*irus* infections in relation to reproductive disorders in dairy cattle in Ethiopia

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ABSTRACT

Reproductive disorders in dairy cattle have been noted to be common in urban and *peri*-urban dairy production system in Ethiopia. The available reports on the causes of these disorders, however, are not conclusive. A case-control study was designed to investigate the possible association of major reproductive disorders in dairy cattle with exposure status to bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV) and Schmallenberg virus (SBV). Cows with history of abortion/stillbirth were considered as cases (n = 204) while, those cows with no such history were taken as control (n = 359). The serological screening tests used for all the three viruses were blocking enzyme linked immunosorbent assays (B-ELISAs). Of the total 563 samples tested 58.4%, 43.8% and 32.9% were positive for SBV, BHV-1 and BVDV, respectively. Significant difference between cases and controls were noted for SBV ($p = 0.026$) and BHV-1 exposures ($p < 0.001$). The difference noted for BVDV serostatus was not significant ($p > 0.05$). The highest proportion (28.9%) of concurrent exposures was noted for BHV-1 and SBV, followed by SBV and BVDV (21.5%) and BHV-1 and BVDV (20.2%). Evidence of exposures to all the three viruses were detected in 14.4% of the animals. However, significant difference between cases (39.7%) and controls (22.9%) among cattle with multiple sero-positivity was noted only for BHV-1 and SBV ($p < 0.001$). Proportion of uterine infection ($p = 0.002$) and fetal membrane retention ($p = 0.005$) increased in BHV-1 seropositive animals, while repeat breeding was common ($p = 0.034$) among BVDV exposed ones. Seropositive animals to any of the three viruses were detected in all sampled areas and the proportion of cattle with BHV-1 and SBV exposure history had a higher risk to at least one type of the reproductive disorders mentioned compared to the corresponding sero-negative groups.

1. Introduction

Improving dairy cattle production has been one of livestock development endeavors for decades in Ethiopia. This has been evidenced with importation of exotic dairy breeds since the early 1950s and later in 1980s artificial insemination service (AI) was introduced for improving local breeds through crossbreeding programs (Ketema, 2000; Ahmed et al., 2004). However, the dairy sector is still underdeveloped for several logistic and technical reasons including the preponderance of infectious diseases that impede the productive and reproductive performance of dairy cattle (Asmare et al., 2013; Bekele et al., 1989).

Infectious diseases affecting reproduction in cattle can create losses all throughout the reproductive cycle by decreasing ovulation, fertilization, embryonic survival, and fetal survival rates (Njiro et al., 2011). Some of such diseases are caused by viruses including infectious bovine rhinotracheitis virus (BHV-1), bovine viral diarrhea virus (BVDV) and Schmallenberg virus (SBV) (Kampa et al., 2004; Anderson, 2011; Pawaiya and Gupta, 2013).

BHV-1 causes diseases commonly known as infectious bovine rhinotracheitis (IBR) or infectious pustularvulvovaginitis (IPV) in cows and infectious pustular balanoposthitis (IPB) in bulls. The infections are contagious and spread through contact with infected cattle shedding

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the virus from respiratory, ocular and reproductive secretions (Anderson, 2011). Bovine herpesvirus –1 (BHV-1) is an alpha-herpesvirus and further characterized genetically into three distinct BHV-l subtypes: BHV-1.1a, BHV-1.1b, and BHV-1.2 (Muylkens et al., 2007; OIE, 2008). Depending on the stage of gestation, infection with BHV-1 can result in early embryonic death and infertility, or late-term abortion (five to nine months of gestation). Fetal mummification and stillbirth are also part of the infection sequel (Radostits et al., 2007; Anderson, 2011).

Preliminary serological surveys conducted in Ethiopia indicated a high prevalence among cattle in different parts of the country. Lefevre (1975) in his first report of IBR recorded a seroprevalence of 42% in Harar and Sidamo provinces located in the eastern and southern part of the country, respectively. The only other report available on IBR in Ethiopia, dating 28 years back, similarly affirmed high level of the infection in Gobe, central highlands (58%) and Ghibe (81%) located in the Ghibe River Valley southwestern areas of the country (Bekele et al., 1989).

Bovine viral diarrhea virus (BVDV) is the other important infectious agent responsible for multiple pathological disorders including reproductive disorders comprising embryonic mortality, abortion, stillbirth, the birth of weak calves and teratogenesis (Van Vuuren, 2005; Al-Afaleq et al., 2007). The virus belongs to the family *Flaviviridae*, genus *Pestivirus* and is segregated into 2 different species, bovine viral diarrhea virus genotype 1 (BVDV-1) and genotype 2 (BVDV-2) (Vilček et al., 2005). Apart from the aforesaid disorders, infection of cattle in the first trimester of pregnancy can result in the birth of a persistently infected (PI) calves that have great epidemiological relevance in the dynamics of BVD (Radostits et al., 2007).

Despite the global distribution and tremendous impact of BVD in the dairy industry (Houe, 2003), the available reports in Ethiopia are very much limited. The first serological evidence of BVD in Ethiopia was reported by Nigussie et al. (2010) in 11.45% of 567 serum samples collected for FMD national surveillance from Oromia Regional State. Later, Asmare et al. (2013), reported BVDV exposure evidence among dairy cattle in central and southern Ethiopia.

Schmallenberg virus was first described in Germany in November 2011 (Hoffmann et al., 2012). Since then it has now been detected in several countries in Europe (EFSA, 2014) the Middle East (Azkur et al., 2013; Abi-Rizik et al., 2017) and China (Zhai et al., 2017) from a variety of host species. SBV infection is noted as one of the emerging infectious diseases of ruminants transmitted by *Culicoides* midges (Pawaiya, and Gupta, 2013). The virus is an enveloped negative-sense, segmented, single-stranded RNA virus, classified in the genus *Orthobunyavirus* of the *Bunyaviridae* family, and is closely related to Akabane, Aino and Shamonda viruses (OIE, 2013). Cattle and other ruminants have been reported to suffer from a range of mild clinical abnormalities manifested by transient fever, anorexia and diarrhea to transplacental transmission in pregnant animals, causing abortions, stillbirths and a variety of congenital malformations mainly involving the skeletal and nervous systems (Gibbens, 2012). In sub-Saharan Africa, studies have documented evidence on possible presence of the virus both in wild and domestic farm animals (Leask et al., 2013; Mathew et al., 2015). A single recently published study, demonstrated a high seroprevalence (56.6%) and a widespread spatial distribution of exposure to SBV in Ethiopia (Sibhat et al., 2018).

The three viruses mentioned above are of different kinds but have a lot in common pertinent to pathologies of reproductive abnormalities including repeat breeding due to embryonic mortality, abortion, stillbirth, fetal mummification, and congenital abnormalities (OIE, 2008; Gibbens, 2012). In developed countries the relative importance of these infectious causes of reproductive disorders has been well documented and respective control regimes have long been underway particularly, for BVDV and BHV-1 (Boelaert et al., 2005; OIE, 2008). Most developing nations are far behind in this regard (Kampa et al., 2004), arguably due to technical and logistic limitation. Likewise, very little is known about the possible cause of reproductive disorder affecting much of the dairy sector in Ethiopia, let alone any control interventions (Asmare, 2014). Reproductive disorders are multifactorial in nature, but the role infectious agents have is tremendous especially where animal health service delivery is inadequate and substandard (Givens, 2006). In this connection, the above-mentioned viral agents are among the leading causes of reproductive wastage in cattle worldwide (Kampa et al., 2004; Anderson, 2011; Pawaiya and Gupta, 2013). Nevertheless, there are only limited evidences available on these agents in Ethiopia. Therefore, this study was undertaken with the aims of investigating the possible associations of bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV) and Schmallenberg virus (SBV) exposures to the reproductive disorders reported and assesses the spatial distribution pattern of the viruses in dairy cattle in major milk sheds of the country.

2. Material and methods

2.1. Study area

This study was conducted in central, south and south-western Ethiopia involving 15 urban and *peri*-urban areas including the capital Addis Ababa. Geographically, the areas are located between 7°03′ to 9°04′ North and 36°50′to 39°27′ East. The conurbations were purposely selected due to establishment of dairy farms in and on their outskirts. The agroecology of the study areas varies from midland where the altitude measures 1700 m above sea level (masl) around Hawassa, an area in the Great East African Rift Valley, to Holleta located in the central highlands at an altitude of 2400 masl. Central and southern Ethiopia milk sheds constitute the two largest and oldest milk sheds that serve as a source of breeding stock for most parts of the country. Jimma town and its adjacent districts are among the emerging urban and *peri*-urban dairy areas and categorized as the western milk shed of the country in the current study.

2.2. Study animals

Dairy cattle composed of Holstein-Friesian, Jersey and their local crosses which are managed intensively or semi-intensively were the target population of the study. Farms were of different kinds ranging from small-scale dairy farms managed with family labor in small settings to public or privately owned large scale farms intended for commercial dairy production and/or breeding purposes.

2.3. Study design

The study was a case-control study aimed at providing an additional evidence as part of an ongoing project that started in 2012 for identifying possible infectious causes of reproductive disorders in dairy cattle in Ethiopia. In line with this a cow was considered as a case based on its specified reproductive disorder history, i.e. having had abortion or stillbirth as retrieved from the farm record where available or owner information where such record is lacking. On the other hand, a control is defined as a cow kept in the same farm but had no history of abortion or stillbirth. In breeding and large-scale farms animal health or production experts were responsible for record keeping. A maximum of two non-matched controls were selected for each case. Matching was intentionally avoided to ensure the availability of controls in small scale urban and *peri*-urban dairy farms. In such farms where the herd size was 10 or less animals, maximum of two cases were considered. For larger farms maximum of 6 cases and 12 controls were sampled. In farms where no sufficient numbers of cows for control existed, heifers above 6 months of age were considered to rule out passively transferred maternal antibodies. Moreover, for all breeding females, the type of reproductive disorder reported in addition to abortion and stillbirth were also recorded.

2.4. Management and operational definitions

Urban and peri-urban dairy: A type of dairy production systems in Ethiopia producing milk either as a full time or a part time business in urban and *peri*-urban areas. These smallholder dairy farms predominantly keep a small number (≤10 animals) of crossbred cows in a zero-grazing system to produce milk for both home use and sale.

Commercial dairy: Farms are also farms located in urban and *peri*-urban areas mainly in and around the major cities and produce milk exclusively for sale. These farms are specialized dairy farms with crossbred and/or pure exotic breeds of dairy cattle (Holstein- Friesian) and are full time businesses with relatively large number (> 10 animals) of animals.

Breeding farms: are farms owned by the government with the primary objective of breed improvement through crossing of indigenous breeds with exotic breeds (Holstein-Friesian and Jersey). The farms supply pregnant cross breed heifers and bulls to rural small holder dairy farms and the National Artificial Insemination Center, respectively.

Abortion/stillbirth: Abortion is a loss of the fetus between 42 and 260 days of gestation, while stillbirth is a calf that was born dead between 260 days and full-term, or died within 24 h following birth (Peter, 2000).

Repeat breeder: A cow that has a history of 3 or more services per conception.

Uterine infection: the detection of any abnormal discharge from the reproductive tract was considered as uterine infection.

2.5. Sample size determination and sampling

The minimum possible sample size for the study was calculated using an online epidemiological calculator (Ausvet, 2012) with the following parameters predetermined, i.e. odds ratio (OR) of 3, an expected prevalence of exposure in control groups of 12% for BVDV (Nigussie et al., 2010), a desired level of confidence of 95%, absolute error of 5%, and 80% power. The minimum calculated sample size to detect the real differences between cases and controls was 101 cases and 202 controls. Due to an expected clustering of cases or herd effect, the sample size was expanded by 25%. Accordingly, the minimum case number was increased to at least 126 with 252 controls. Following determination of the minimum possible samples size, the sampling frame of herds in each conurbation was prepared in collaboration with respective animal health departments. In each conurbation, a minimum of 10% of herds in the list were considered for the study and farms were randomly selected. In a situation where the chosen farm had no cow with such history, the immediate nearest farm was used as a replacement. In farms where large numbers of cases were available, separate lists were prepared both for cases and controls and individual animals were randomly selected. Otherwise, the available case and controls were all considered as per the predefined inclusion criteria. Finally, blood samples and relevant individual animal biodata were collected on 563 animals of which 204 were cases and the remaining 359 were controls. Altogether, animals were sampled from 110 farms in 15 conurbations located in three geographic regions of the country.

2.6. Blood sampling

Seven to ten milliliters of blood was collected either from the jugular vein or coccygeal artery aseptically using sterile needles and vacutainer tubes. The blood samples were allowed to stand overnight at room temperature before being centrifuged at 1000*g* for 10 min. Serum from each sampled animal was transferred to a sterile cryovials. Each of the samples were labeled with unique codes that corresponds to farm and individual animal identification codes. The samples were then transported to National Veterinary Institute, Bishoftu (Debre-Zeit), Ethiopia in an ice box and kept at −20 °C until they were tested.

2.7. Serological assay

Blocking enzyme linked immunosorbent assay (B-ELISA), (INgezim IBR Compac, Ingenasa, Madrid, Spain) which uses an HRPO-labeled monoclonal antibody specific to Infectious Bovine Rhinotracheitis Virus gB protein as a conjugate, was used to detect antibodies specific to BHV-1 according to the manufacturer's protocol. Briefly, 50 µ1 diluent (a mxture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) was added to all wells and then 50 µ1 sera (test samples, negative and positive control) were added as per plate layout into BHV-1 antigen pre-coated plate. The plate was incubated for an hour at 37 °C and washed five times. Then 100 µ1 of conjugate was added to all wells and plates were incubated at 37 °C for 30 min and washed out. Finally, 100 µ1 of substrate solution was added, kept at room temperature in a dark place for 15 min, stopped by 100 µ1 stop solution (0.16 M sulfuric acid) and OD value was read with ELISA reader at 450 nm. The blocking percentage was calculated and samples with% Block ≥ 30% was considered as positive. In blocking ELISA, conjugated monoclonal antibodies bind to the viral specific antigen when the test serum is negative for antibodies against natural infection or vaccination. However, since there has never been any history of vaccination for any of the three viruses mentioned in the study area, authors believe animals' seropositivity were due to natural infections.

For Schmallenberg virus also a blocking ELISA (INgezim SBV Compac, Ingenasa, Madrid, Spain) was used. Briefly, 90 µ1 diluent was added to all wells and then 10 µ1 sera (test samples, negative and positive control) were added as per plate layout into SBV antigen pre-coated plate. The plate was incubated for an hour at room temperature, washed five times, and then 100 µ1 of conjugate (HRPO-labeled monoclonal anti-SBV antibody specific to the N protein of the virus) was added to all wells, incubated for half an hour at room temperature and washed out. Finally, 100 µ1 of substrate solution was added, kept at room temperature in a dark place for 15 min, stopped by 100 µ1 stop solution and OD value was read with ELISA reader at 450 nm. The blocking percentage was calculated and samples with% Block ≥ 55% was considered as positive.

The presence of antibodies to BVDV non-structural p80 protein was determined using a blocking ELISA (INgezim Pestivirus Compac, Ingenasa, Madrid, Spain) that uses HRPO-labeled monoclonal antibodies against p80 protein of BVDV as a conjugate. In short, 100 µ1 of positive, negative and test sera (pre-diluted 1/5) were dispensed to respective wells of pre-coated plate. The plate was incubated for an hour at 37 °C, then, without removing serum samples, 50 µ1 conjugate was added, shaken to homogenize and incubated at room temperature for an hour. The plate was washed five times. Finally, 100 µ1 of substrate solution was added, kept at room temperature in a dark place for 15 min, stopped by 100 µ1 stop solution and OD value was read with ELISA reader at 450 nm. The positive and negative cut-off was calculated and test sample result was determined based on the cut-off value.

2.8. Data management and analysis

A database was generated in Excel® (version, 2010). Following cleaning and editing, the data were transferred to Stata SE/14 for Windows (STATA, Stata Corp. LP, 4905, Lakeway drive, College Station, Texas, USA). Preliminary summary, i.e. frequencies and proportions were computed for both dependent and independent variables. Univariable logistic regression analysis was done to assess the presence or absence of statically significant difference ($p < 0.05$) between cases and controls. Similar analysis was computed for concurrent serological profile versus the predefined reproductive disorders. Reproductive abnormalities other than abortion and stillbirth were separately assessed using unconditional univariable logistic regression analysis by considering serostatus of each viral infection as the independent and occurrence of the reproductive disorder as dependent variables. A Venn diagram and a web chart were also used to illustrate the level of co-

Table 1
Serological Evidence of multiple viral exposures in relation to cases and controls.

Type of virus	Proportion of seropositives			OR (95% CI)	p-value
	Total sample size (N = 536)	Cases (n_1 = 204)	Controls (n_2 = 359)		
SBV	58.4	64.7	55.0	1.5 (1.1, 1.9)	0.026
BHV-1	43.8	55.4	37.8	2.0 (1.4, 2.9)	< 0.001
BVDV	32.9	36.3	31.5	1.2 (0.9, 1.8)	0.252

SBV = Schmallenberg virus, BHV–1 = Bovine herpesvirus-1, BVDV = Bovine viral diarrhea virus.

infection and proportion of spatial distribution, respectively.

3. Results

In this study a total of 563 animals were sampled, and 58.4% of them were positive for SBV exposure. Similarly, 43.8% and 32.9% were the proportion of sero-reactors for BHV-1 and BVDV, respectively. The presence of statistically significant differences ($p < 0.05$) between cases and controls were noted for SBV and IBR exposure, while no statistical difference was documented for BVDV exposure (Table 1).

3.1. Serological imprint for concurrent infection

The available serological evidence for concurrent infection with two or three of the viruses was common. The concurrent serological imprint for BHV-1 and SBV was the highest (28.9%) followed by SBV and BVDV (21.5%). In 14.4% of the animals, exposure evidence to all the three viruses was detected. However, statistically significant difference between cases and controls was noted for co-infection with BHV-1 and SBV exposure history (Table 2). The graphical overlap for the three of viruses is given in Fig. 1.

3.2. Association with other reproductive disorders

Apart from abortion and stillbirth, the respective exposure statuses of each of the three viruses were assessed against repeat breeding, uterine infection, retention of fetal membranes, birth of weak and congenitally defective calf. In this regard, the proportion of animals with uterine infection and fetal membrane retention were frequently found in cattle seropositive to BHV-1 exposure, while repeat breeding was noted more commonly among BVDV exposed animals (Table 3).

3.3. Spatial distribution

The spatial distribution patterns of sero-reactors' proportions in the study areas were summarized in Fig. 2. Exposure evidence to the three viruses were detected in all the sampled conurbations. The highest proportion of SBV sero-reactors (96.5%) were observed at Wendo-Genet followed by Hawassa (86.1%). BVDV sero-positives were frequently encountered around Holleta (64.3%), Jimma (47.1%) and Ambo (42.2%). Allage, Jimma and Shashemene on the other hand had 89.5%, 68.2% and 66.7% BHV-1 sero- reactors, respectively.

Table 2
Serological evidence of viral co-infection profile between cases and controls.

Types of virus	Overall proportion	Prop. in cases	Prop. in controls	OR (95%CI)	p-value
BHV-1 and SBV	28.9	39.7	22.9	2.2 (1.5,3.2)	< 0.001
SBV and BVDV	21.5	24.5	20.0	1.3 (0.9,1.8)	0.221
BHV-1 and BVDV	20.2	20.1	20.6	1.0 (0.6,1.4)	0.881
BHV-1, BVDV and SBV	14.4	15.6	13.7	1.2 (0.7,1.8)	0.533

Prop. = proportion, OR = odds ratio, CI = confidence interval.

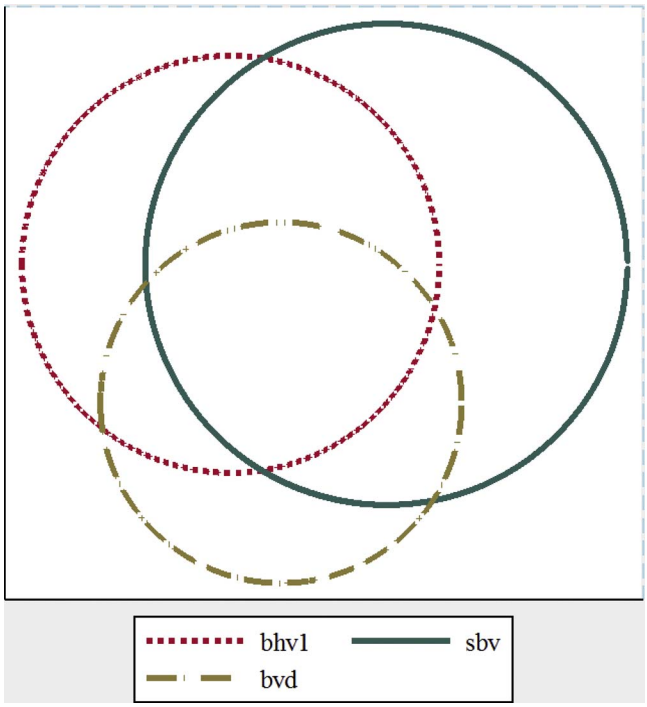

Fig. 1. Venn-diagram of concurrent exposure pattern among BHV-1, BVDV and SBV. In the figure above the extent of single and multiple viral exposure overlap is illustrated which warrant the need for multiple agent assessment in the effort of reproductive disorder diagnosis.

Table 3
Specific reproductive disorders versus infectious agents' exposure profile (n = 536).

Type of reproductive disorders reported	Category	Frequency	SBV (*p*-value)	BHV-1 (*p*-value)	BVD (*p*-value)
Repeat breeding	No	346	0.895	0.149	0.034
	Yes	188			
Uterine infection	No	320	0.417	0.002	0.211
	Yes	218			
Fetal membrane retention	No	326	0.305	0.005	0.542
	Yes	208			
Birth of weak calf	No	525	0.842	0.059	0.508
	Yes	9			
Birth of congenitally defective calf	No	410	0.661	0.281	0.378
	Yes	15			

4. Discussion

This study revealed the presence of SBV, BHV-1 and BVDV sero-reactors among breeding and dairy cattle in the study areas with high proportions. To authors' knowledge, there are few serological evidences available on the possible presence of these viruses in Ethiopia (Lefevre,

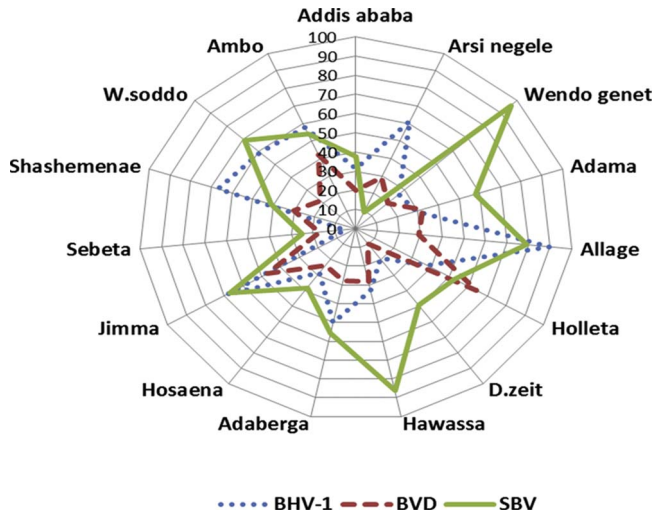

Fig. 2. Proportion of sero-reactors in relation to study areas. (D. Zeit = Debre Zeit, W. sodo = Wolaita Sodo).
The web diagram illustrates how widely sero-reactor cattle are distributed in the study areas. It would be inappropriate to consider the proportion given for prevalence estimate as the design is case control.

1975; Bekele et al., 1989; Nigussie et al., 2010; Sibhat et al., 2018), and the risks of reproductive disorders attributed to their exposure is unknown or indistinct. In this study, it was found out exposure to SBV and BHV-1 were more frequently associated with dairy cows that had history of abortion and stillbirth as compared to cows that had neither. However, no such differences were noted for BVDV exposure between groups compared. The later finding on BVDV exposure corroborates with reports of Asmare et al. (2013) where comparison was made for *N. caninum*, *Brucella* spp. and BVDV exposure between cases and controls. Likewise, in studies conducted in southern Africa that focused on the clinical effects of BVDV, infections in dairy herds didn't associate with abortion (Van Vuuren, 2005). Perhaps this may be explained by the epidemiology of BVDV in that the infection is highly contagious and the within herd prevalence could rise to over 60% in a short time and most animals in transient infection clear the virus and remain with solid immunity for an extended period (Houe, 2003; Talafha et al., 2009). In such scenario, controls taken from the same herd have high probability of seropositive status. The preceding fact might have obscured the anticipated difference between our cases and controls as there is no recurrence in BVDV like IBR associated with stress (Ackermann et al., 1982; Jones and Chowdhury, 2008).

The report of SBV exposure at observed proportion and its association with reproductive disorders complies with reported pathology of the virus on naïve pregnant cow in Europe (Hoffmann et al., 2012). Studies conducted following the outbreaks in various countries showed the impact of the virus on reproductive performance of cattle to be limited at least at the national or provincial levels (Toson et al., 2015; Wüthrich et al., 2016). In this connection, there is a need for further evidence in Ethiopia. It should be remembered that clinical and serological evidence of exposure to Schmallenberg and other related viruses in African continent existed prior to the 2011 SBV outbreak in Europe (Leask et al., 2013; Mathew et al., 2015).

For BHV-1, in addition to abortion and stillbirth, other reproductive disorders including repeat breeding, retention of fetal membranes, uterine infection, and birth of weak and congenitally defective calves were also assessed. Accordingly, animals with history of uterine infection and retention of fetal membrane were frequently sero-positive to BHV-1, while cows with history of birth of weak calves had marginally higher exposure compared to their counterparts. This observation complies with widely known effect of BHV-1 infection on dairy cattle that causes necrotizing chronic endometritis and retention of fetal membranes (Graham, 2013).

Despite the lack of significant difference on case versus control comparison, BVDV exposure was significantly higher among repeat breeders. In corroboration with our finding, a study conducted in Turkey on 139 repeat breeding cows found BVDV to be the cause in 58.2% of the cows (Gür, 2011). In South Africa, Van Vuuren, (2005) also demonstrated the frequent occurrence of repeat breeders in several herds with confirmed BVDV infections. Repeat breeding together with early embryonic death and infertility in cows were some of the sequela of *Pestivirus* infection during pregnancy (Brownlie et al., 2000).

Regarding the spatial distribution, except for SBV at Sebeta and BVDV at Debre Zeit (Bishoftu), exposure to all the three viruses were observed to prevail between 17–96% among sampled animals in all areas. This evidence is enough to disclose the presence, widespread geographic distribution and high risk of exposure of dairy cattle to the three viral infections. Nevertheless, as the study design and laboratory test used were case-control and serology, respectively, it is beyond the scope of the study approach to extrapolate the percentage reported into prevalence of infection.

The other important observation noted in this study was the presence of large number of concurrent –reactors to multiple agents. Besides, BHV-1 and SBV exposure was significantly associated with stillbirth and abortions. Such associations have not been previously observed anywhere else. On the other hand, a previously observed synergistic association between BHV-1 and BVDV sero-positivity (Biuk-Rudan et al., 1999) was not observed in the current study. This could be due to infections with the viruses occurring during different periods or it could be the result of infection when the cows were not pregnant and the serological imprint accumulate over a considerable period. Perhaps, infection might have occurred before or after the reproductive disorders of interest, as the viruses appear to be endemic in the study areas. A clearer picture for the relative importance of such co-infections might be obtained through identification of the causative agents and associated pathologies in longitudinal studies. Proportionally, similar levels (20.65%) of co-detection of antibodies to BHV-1 and BVDV was reported in a previous study from Turkey (Aslan et al., 2015).

In the light of the scarce evidence on cause of reproductive disorders in cattle in Ethiopia, this report provided an important insight into the problems; however, it is difficult to conclude that all the disorders in every animal to be attributed to a specific agent to which the animal was seropositive. This is partly because of the possibility of occurrence of abortion or any of the other disorders for plenty of other reasons not mentioned here, while having the infection with agents under investigation. It could also be possible that the specific animal got infection after the occurrence of disorder was reported or recorded by the farmer. This is part of the inherent limitations of the study design chosen as it does not show temporal sequence of events under investigation.

Finally this study emphasizes that, BHV-1, and SBV are among the important possible causes of abortion and stillbirth in dairy cattle in Ethiopia. Moreover the BHV-1 exposure was noted to associate with uterine infection and retention of fetal membrane, while BVDV with repeat breeding. Thus authors would like to recommend the need for in-depth study on these and other infectious causes of reproductive disorders to launch strategic intervention considering both the economic and public health importance of diseases affecting the dairy sector.

Conflict of interest

None

Author's contribution

KAs designed the study, did the field works, analyzed data and drafted the manuscript. BS and KAr participated in write-up. GA did the laboratory works and participated in write-up. EZ took part in field

works and participated in write-up. ES supervised the study, data analysis, interpretation and enriched the manuscript. All authors have read and approved the manuscript.

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