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Seroprevalence of Schmallenberg virus in dairy cattle in Ethiopia

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ABSTRACT

Schmallenberg virus (SBV) is a recently identified member of the genus *Orthobunyavirus* of the family *Bunyaviridae*. It is an arbovirus transmitted by different members of *Culicoides* spp of biting midges. The virus is more recognized for its effect on reproductive disorders in ruminants characterised by abortion, stillbirth and birth of congenitally defective newborns with hydranencephaly-arthrogryposis syndrome. The current study was undertaken with the objectives of exploring the presence of SBV exposure and identification of factors affecting its distribution among dairy cattle in Ethiopia. A cross-sectional study was conducted on 1379 dairy cattle sampled from 149 dairy herds in central, southern and western Ethiopia during September 2011 to May 2012. Serum samples were examined using competitive enzyme linked immunosorbent assay (cELISA). Data on hypothesised risk factors were collected from farm records where available and semi-structured questionnaire-based interview. The apparent seroprevalence of exposure to SBV was 56.6% (95% confidence interval (CI): 53.9–59.3). True prevalence adjusted for sensitivity and specificity of the cELISA kit used was 58.3% (95% CI 55.7–60.9). Among the sampled herds, 82.6% (95% CI: 75.5–88.3) had at least one seropositive animal. Seropositive cattle were found in all of the 15 conurbations studied. Adult dairy cows [odds ratio (OR) = 1.6] were more commonly affected than young heifers. Dairy cattle kept in commercial (OR = 1.6) and breeding farms (OR = 3.5) and Midland agroecology (OR = 2.5) showed statistically significant seroconversion than cattle kept under small-holder dairy farms and Highland agroecology respectively ($p < 0.05$). Reproductive disorders including abortion, retention of the fetal membranes, and metritis were associated with serostatus of SBV. In conclusion, the seroprevalence of SBV is high and widely distributed in the studied parts of Ethiopia. This being the first study of its kind on SBV in Ethiopia, further longitudinal studies on isolation of the virus and its impact on reproductive disorders are recommended.

1. Introduction

Schmallenberg virus (SBV) is a new virus identified in Germany during the summer of 2011 through metagenomic analysis. The samples for the analysis were taken from dairy cattle affected by a transient fever, diarrhoea and drop in milk production in the absence of other causative agents. The virus belongs to the family *Bunyaviridae* (currently renamed as *Peribunyaviridae*, (ICTV, 2017)) and genus *Orthobunyavirus*. It is a member of Simbu serogroup with other related viruses including Shamonda, Sathuperi, Douglas, Akabane and Aino viruses (Hoffmann et al., 2012). Members of the *Orthobunyavirus* are arthropod-borne (arboviruses) viruses transmitted primarily by *Culicoides* spp. (De Regge et al., 2012; Elbers et al., 2013; European Food Safety Authority (EFSA, 2014)). Different groups of *Culicoides* associated with the transmission of SBV in Europe during the outbreak of the virus in 2011/12 include *Culicoides obsoletus* complex, *C. chiopetrus* and *C. dewulfi* (Doceul et al., 2013).

Apparent clinical signs of SBV infection in adult cattle are reported to be short-lived. These include loss of appetite, hyperthermia, diarrhoea, and reduction in milk production. Infection during the certain critical period of pregnancy between days 47 and 162 of gestation (Wernike et al., 2014), was shown to cause neonatal malformation affecting neuro-musculo-skeletal systems (Hoffmann et al., 2012; Beer et al., 2013). The syndrome is known as arthrogryposis

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hydraenchphally (AHS) and characterised by arthrogryposis, severe torticollis, ankylosis, kyphosis, lordosis, scoliosis, brachygnathia inferior and neurological disorders. Most of the anomalies were observed in cases of abortions and stillbirths, while some calves may be born alive with various pathologies and behavioural abnormalities (Garigliany et al., 2012a). Apart from the reproductive disorders and transient clinical disease that affected adult cattle, the majority of infections were in-apparent. In a study by Veldhuis et al. (2014), seropositivity to SBV was mildly but significantly associated with decreased reproductive performances in the Netherlands and Germany. Since the report of Hoffmann et al. (2012), serological and/or molecular evidence of the virus were available from several European countries including Belgium, France, Greece, UK, Italy, Spain, Luxembourg, Denmark, Poland, Sweden and Switzerland (Beer et al., 2013; EFSA, 2014); Turkey (Azkur et al., 2013; Tonbak et al., 2016) and China (Zhai et al., 2017).

In Africa, the available research evidences on SBV in cattle are few; however, seroprevalence as high as 61% in Tanzania (Mathew et al., 2015) and 100% in Mozambique (Blomström et al., 2014) were reported. To the authors' knowledge, no research report is yet available on SBV existence in Ethiopia. Nevertheless, given the attributed SBV pathology (Gibbens, 2012; Luttikholt et al., 2014) along with the high proportion of un-identified reproductive disorders in cattle in Ethiopia (Asmare, 2014), it is logical and reasonable to assess the potential role of SBV (if any) as one of the possible causes of the syndromes in question in the country. Thus, this study was conducted with the objectives of exploring the existence, seroprevalence and possible association of SBV exposure with reproductive disorders in cattle in Ethiopia. The serum samples in use were obtained from an ongoing project on the main causes of reproductive disorders which were collected during September 2011 to May 2012.

2. Materials and methods

2.1. Study area

The study was conducted in 15 dairy production potential areas where the relatively long history of keeping improved dairy breeds in Ethiopia prevails. These include Addis Ababa, Adama, Ambo, Sebeta, Holeta, Bishoftu and Adda Berga in central milkshed; Arsi Negele, Allage, Shashemene, Hawassa, Wondo-Genet, Hosaena and Wolita Sodo in southern milkshed and Jimma and its surroundings in the western milk shed. Milkshed refers to areas that supply milk and other dairy products to major urban population centres in parts of the country they represented.

2.2. Study animals

The study animals were Holstein-Friesians (HF), Jersey and HF-Zebu crossbred cattle reared in small-holder and commercial dairy farms located within and on the outskirts of aforementioned major towns. The study also included four government-owned breeding farms. In this study, small-holder, dairy farms are those holdings up to 10 dairy cattle. These farms produce milk for household consumption and commercial purposes, while commercial dairy farms are the ones with more than ten dairy cows and produce milk basically for sale. Breeding farms are large farms established by the government for dairy improvement through crossbreeding of exotic cattle (Holstein-Friesians and Jersey) with local zebu. The objectives of such farms are to improve the dairy sector through the distribution of pregnant crossbred heifers to rural small-holder dairy farmers at subsidised prices. One of the breeding farms located at Holeta is a bull-dam station raising bulls for the National Artificial Insemination Center, a semen production facility that distributes semen across the country.

2.3. Study design

Serum samples used in this investigation (n = 1379) were collected during September 2011 to May 2012 using a cross-sectional study design to screen dairy cattle in central, western and southern areas of the country for major infectious causes of reproductive disorders including bovine herpesvirus 1 (BHV-1), *Neospora caninum, Brucella* spp. and bovine viral diarrhoea (BVD). Exposure to SBV, however, was not part of the study at that time. Therefore, the descriptions hereunder explain how the samples were originally sourced.

The study sites were selected purposively based on the reasons described under the study animals' sub-section, while farms were selected randomly from a list of dairy farms produced by the support from veterinary officers and livestock production professionals at agricultural offices in the respective districts where the towns are located. A minimum of 10% of dairy farms was selected from each conurbation. Individual animals within the herds were also selected based on random sampling using a lottery method. Two to 75 animals were chosen from each farm depending on herd size representing at least 10% of the dairy cattle between the ages of six months and above. Four breeding farms were selected purposively. Overall, 149 herds consisting of 125 small-holder, 20 commercial and four breeding farms were included in the study.

2.4. Sampling and sample size

Among infectious causes of reproductive disorders previously reported from dairy cattle in Ethiopia, the highest prevalence was reported for exposure to BHV-1. The sample size was determined based on a formula for simple random sampling (Thrusfield, 2007) for BHV-1 exposure based on a prior prevalence of 67% (Bekele et al., 1989), 5% absolute precision and 95% confidence level. Accordingly, the minimum sample size required to estimate the prevalence of the disease was calculated to be 339 animals. The distribution of dairy animals in Ethiopia is not uniform as the history and experience of keeping improved dairy cattle differ from one area to the other. Therefore, the samples were proportionally allocated to the three geographic regions; 50% (n = 169), 40% (n = 136) and 10% (n = 34) respectively, to central, southern and western milk-sheds based on livestock data obtained from the Central Statistical Agency (CSA, 2011). As the primary sampling units were herds, to account for the design effects and diseases for which prior prevalence was not known, the sample size was expanded four times, and 1379 serum samples were collected from 149 herds. Geographically, 555, 629 and 195 dairy cattle were sampled from central, southern and western Ethiopia, respectively.

2.5. Serological assays

The serum samples collected were kept stored at −20 °C at the National Veterinary Institute, Bishoftu (Debre Zeit), Ethiopia. A multi-species enzymatic immunoassay based on a blocking ELISA technique, which uses a monoclonal antibody specific to the N protein of Schmallenberg virus (INgezim SBV Compac, Ingenasa, Spain) was used to screen the sera for the presence of blocking anti-SBV antibodies following manufacturer's procedures. Briefly, 90 µ1 diluents (a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) 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 the conjugate 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 (0.16 M sulfuric acid), and OD values were read with ELISA reader at 450 nm. The blocking percentage was calculated, and samples with% B > 55% were considered as positive. The ELISA kit used had

98% sensitivity and 99% specificity according to the validation tests conducted by the manufacturer. All the required reagents and a 96 well microtitration plate were provided ready to use within each purchased package.

2.6. Data analysis

During blood sampling data were collected from dairy farm records in farms that keep individual animal records, and from farm managers/owners through a semi-structured questionnaire administered by one of the investigators in farms where records were not available. Both individual and herd level data collected during sampling were entered into Microsoft Excel spreadsheet. The following were predictor variables collected from each farm: type of farm categorised as smallholder, commercial and breeding; herd size as small (5–10 dairy cattle) and large (> 11 cattle in the farm). Breed of animals kept in the farms as Holstein-Friesian, Jersey, or Holstein-Friesian X Zebu cross. Age of the animals was categorised as young (6 months to < 17 months) and adult (17 months and above). Origin of the individual animal was classified either as 'homebred' when born on the same farm or 'purchased' when introduced from other farms. Presence of reproductive disorders, i.e. abortion, stillbirth, retained fetal membranes (RFM), the birth of weak and congenitally defective calf each as 'Yes' when present or 'No' otherwise. The mean calving interval was considered as 'Expected' when the next calving occurred between 12 and 18 months or 'Prolonged' if longer than 18 months. Geographic region of the farm location was registered as central, southern or western Ethiopia; and agroecology of the area was classified as Midland at an altitude of 1600–2000 m above sea level (masl) or Highland (altitude > 2000 masl)

The data was cleaned, coded and imported to STATA release 14.0 software (Stata Corp., College Station, Texas) for further statistical analyses. Individual animal level SBV apparent seroprevalence (AP) was computed using the survey command with cluster (herd) adjustment and sampling weight. True prevalence (TP) of SBV exposure was calculated by adjusting the apparent prevalence of specificity and sensitivity of the test using the formula, TP = AP + (Sp-1)/Se + (SP-1), where AP is apparent prevalence, Sp is specificity and Se is sensitivity of the blocking ELISA test used (Dohoo et al., 2009). The design effect was calculated using the formula, D = 1 + (k-1)ρ, where, k is the average number of animals sampled from the herds (k = 9) and, ρ, the intra-herd correlation coefficient from the one way random effect model, was calculated using the command '*estat*' effects in STATA 14.0 following model building.

Associations between individual animal serostatus and predictors and possible consequences of infection with SBV were assessed using univariable logistic regression. All predictors were checked for multicollinearity in a cross-tabulation using Goodman and Kruskal's Gamma statistic. In the development of the multivariable logistic regression model, all the non-collinear predictors (gamma values, −0.6 to +0.6) with generous p-values of 0.2 and lower were considered, and the final model was developed using backward elimination technique based on Wald's test and likelihood ratio test statistics ($p < 0.05$). The interactions between predictors were tested by constructing two-product terms for the significant main effect variables, forcing them into the model and examining changes in OR and p-values of the main effects. The changes in the proportion of OR were also used to check for confounding effect. A covariate was considered to be a confounder and included in the model if its inclusion altered the OR of the estimated risk a minimum by 20%. The final model was assessed using the Hosmer and Lemeshow method for goodness-of-fit and the receiver operating curve (ROC) for reliability (Dohoo et al., 2009). Apparent prevalence of SBV in the sampling sites were presented using a proportional dot map generated using ArcGIS version 10.4.1 software (ESRI, Redlands, California, USA)

Table 1
Herd and animal level seroprevalence of SBV exposure in the three major milksheds of Ethiopia.

Part of the country	Animal level		Herd level	
	No. Sampled	Prev.(95%CI)	No. Sampled	Prev. (95% CI)
Central Ethiopia	555	49.7 (45.6–53.9)	53	84.9 (72.4–93.3)
Southern Ethiopia	629	61.2 (57.3–64.9)	77	76.6 (65.6–85.5)
Western Ethiopia	195	61.0 (54.0–67.6)	19	100 (82.4–100)*
Overall	1379	56.6 (53.9–59.2)	149	82.6 (75.5–88.3)

No. = Number, Prev. = Prevalence, *binomial distribution.

3. Results

3.1. Seroprevalence

Among 1379 serum samples screened for SBV exposure 780 (56.6%) dairy animals tested positive. The true prevalence adjusted for the sensitivity and specificity of the diagnostic test was 58.3% with 95% confidence interval (CI) of 55.7–60.9%. About geographic regions, central Ethiopia had a lower prevalence of exposure as compared to both southern and western Ethiopia. The differences, however, are not statistically significant ($p > 0.05$). Herd-level seroprevalence was 82.6% with all sampled herds in western Ethiopia testing positive for exposure to SBV (Table 1). Seropositive cattle were found in all the 15 sampled conurbations with seroprevalence ranging from 7.4% at Arsi Negele to 100% at Wondo-Genet (Fig. 1).

3.2. Risk factors

Univariable logistic regression analysis indicated that seroprevalence to SBV might be affected by the type of farm, agroecology, herd size, breed and age of sampled animals. However, geographic location of the farms and origin of the dairy animals were not found to be associated with seropositivity ($p > 0.05$) (Table 2).

For multivariable logistic regression analysis, collinear predictors, namely, herd category and origin of cattle were dropped from the model. All the remaining non-collinear variables were used to build the final model, and three predictors were found to be significantly associated with seroprevalence of SBV exposure($p < 0.05$). Accordingly, commercial and breeding farms had more seropositive animals as compared to small-holders. Cattle kept in Midland agroecology, and cows 17 months of age and above were also noted to have higher seroprevalence than their corresponding categories (Table 3).

In a univariable logistic regression analysis of presumed consequence of viral exposure, significant associations ($p < 0.05$) were noted for abortion and its increasing frequency, metritis and retention of fetal membrane (Table 4). Cows that tested positive for SBV antibodies had a higher proportion of reproductive disorders as compared to seronegative ones (Fig. 2).

3.3. Intra-herd correlation coefficient and design effect

The intra-herd correlation coefficient and design effect calculated for the SBV exposure were 0.13 and 2.04 respectively.

4. Discussions

Anti-SBV antibodies were highly prevalent both at an individual animal (56.6%) and herd level (82.9%). Evidence of the viral exposure was noted in all sampled sites and the proportion of seropositive cattle ranged from 7.5% at Arsi Negele to 100% at Wondo-Genet. The

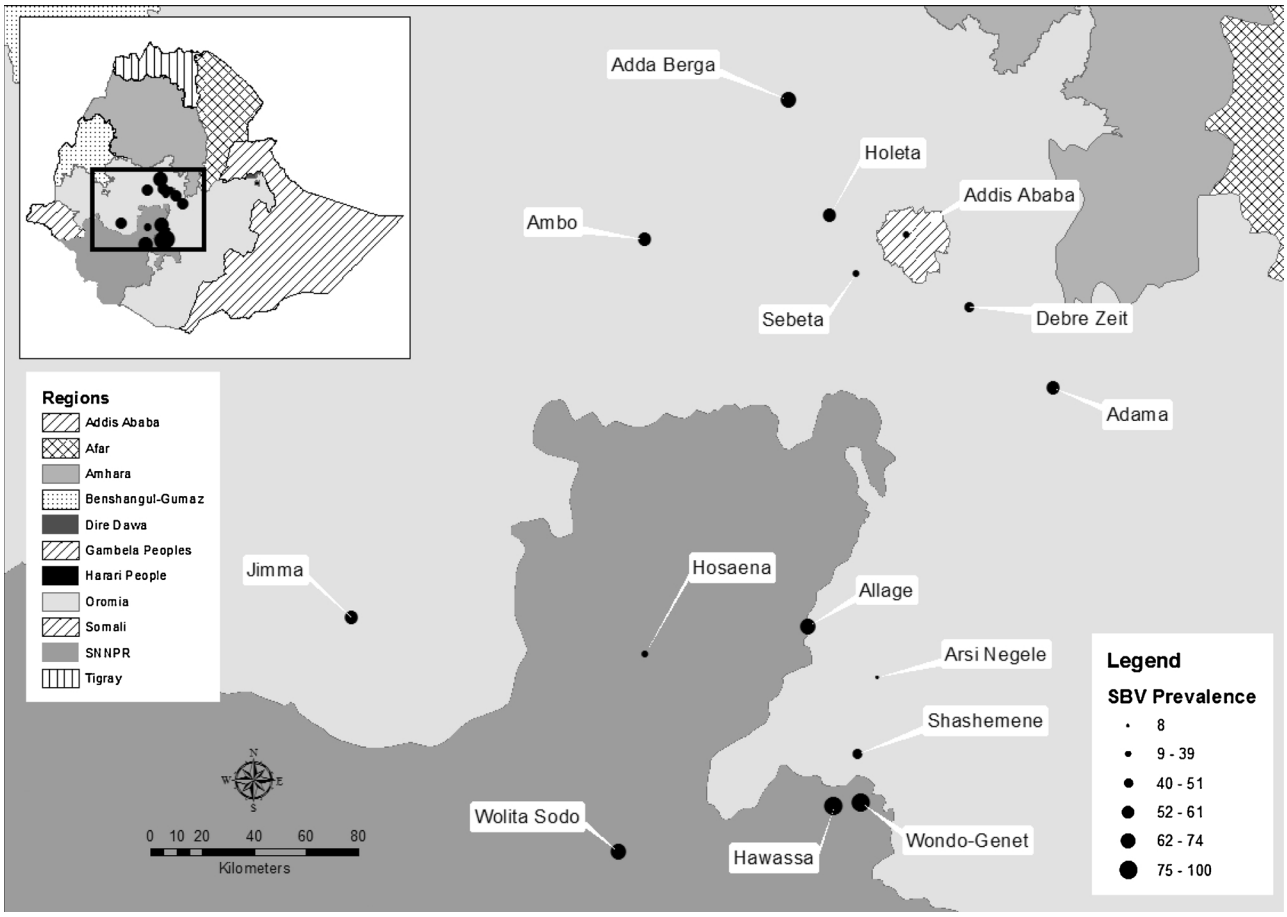

Fig. 1. Distribution of Schmallenberg virus antibodies in three geographic regions of Ethiopia. The areas were Addis Ababa, Adda Berga, Adama, Ambo, Debre-Zeit (Bishoftu), Holeta and Sebeta from Central Ethiopia; Allage, Arsi Negele, Hawassa, Hosaena, Shashemene, Wolaita Sodo and Wondo-Genet from southern and Jimma and its surroundings from western Ethiopia.

observed high prevalence along with distribution may imply that the virus might have been in the country for some time before the outbreaks of the disease in northern Europe. This finding may corroborate with a report from Tanzania where the seroprevalence of SBV antibodies was reported to reach as high as 61%. The same study also reported the existence of exposure to the virus in Africa before 2011 (Mathew et al., 2015). In another study, higher seroprevalence (100%) was reported from Mozambique (Blomström et al., 2014). Although the overall seroprevalence report in the current study was lower than the Mozambican study, some of the study sites in Ethiopia such as Hawassa (88%) and Wondo-Genet (100%) had similar high seroprevalence indicating that ecological factors might dictate such differences. Similarly, seroprevalence of exposure to SBV, 24.5% and 57.4% were reported from cattle in Turkey (Azkur et al., 2013) and China (Zhai et al.,

Table 2
Univariable logistic regression analysis of the presumed risk factors in relation to SBV exposure status among dairy cattle in central, southern and western Ethiopia.

Variables	Categories	No. examined	Prev. (95% CI)	OR (95% CI)	p-value
Type of farm[a]	Small-holder	233	53.6 (49.2–57.9)	Reference	–
	Commercial	904	54.2 (50.3–58.0)	1.04 (0.8–1.3)	0.739
	Breeding	242	69.0 (62.9–74.5)	1.2 (0.8–1.8)	0.294
Geographic region	Central	555	49.7 (45.6–53.9)	Reference	–
	Southern	629	61.2 (57.3–64.9)	1.6 (1.3–2.1)	< 0.001
	Western	195	61.0 (54.0–67.6)	1.7 (1.2–2.5)	0.002
Agro-ecology[b]	Highland	525	62.6 (59.3–65.8)	Reference	–
	Midland	854	46.7 (42.4–50.9)	1.6 (1.2–2.1)	< 0.001
Herd category[a,b,c]	Small (< 10)	233	44.2 (37.9–50.7)	Reference	–
	Large (> 10)	1146	59.1 (56.2–61.9)	1.7 (1.3–2.3)	< 0.001
Breed[c]	HF	1055	53.1 (50.1–56.1)	Reference	–
	HF-zebu cross	170	60 (52.4–67.1)	0.8 (0.5–1.2)	0.237
	Jersey	150	76 (68.5–82.2)	2.4 (1.6–3.7)	< 0.001
	Local	4	100 (36.8–100)*	1	–
Age[d]	Young	210	48.1 (41.4–54.9)	Reference	–
	Adult	1169	58.1 (55.2–60.9)	1.8 (1.2–2.5)	0.002
Origin	Homebred	757	55.5 (51.9–58.1)	Reference	–
	Purchased	594	57.4 (53.4–61.3)	1.2 (0.9–1.5)	0.234

OR = Odds Ration, HF = Holstein-Frisian, Prev. = Prevalence, *binomial exact one sided 97.5% CI, a,c,b,d = varaibles with similar superscripts are collinear.

Table 3
Multivariable logistic regression model for potential predictors of SBV seropositivity in dairy cattle in selected geographic regions of Ethiopia.

Variables	Categories	Odds Ratio (95% CI)	p-value
Type of farm	Small-holder	Reference	–
	Commercial	1.6 (1.2–2.1)	0.001
	Breeding	3.5 (2.4–5.5)	< 0.001
Agroecology	Highland	Reference	–
	Midland	2.5 (2.0–4.0)	< 0.001
Age	Young	Reference	–
	Adult	1.6 (1.1–2.1)	0.008

Hosmer-Lemeshow χ^2 = 8.88, p-value = 0.1134, ROC = 0.6368

2017), respectively.

The seroprevalence was shown to be affected by the type of farm; commercial and breeding farms were more at-risk of having seropositive animals than small-holder farms. These differences might have arisen from unhygienic waste management in larger commercial dairy and breeding farms where the wastes are dumped into open pits or hipped up in farm vicinity. On the contrary, such practice is rare in small-holder farms where the dung is dried and made into a product that is burned as a source of energy replacing firewood for cooking in most dairy farming areas of the country (Negash et al., 2017). Moist soils rich in organic matter such as those created by dumping manure and other farm wastes may well support the breeding of *Culicoides* spp. (Constable et al., 2017).

Agroecological differences between the locations of the farms had a significant effect on seroprevalence of SBV. Farms located in Midland areas had higher seroprevalence than those located in the highlands ($p < 0.05$). This could be associated with favourable environmental conditions created by factors including warmer average temperatures and moisture that support insect breeding and multiplications in the low-lying areas (Constable et al., 2017). In Ethiopia, other diseases transmitted by *Culicoides* species, such as African horse sickness and bluetongue were shown to be more prevalent in the lower altitudes representing lowland and midland agro-ecologies than the cool highlands (Woldemeskel et al., 2000; Kassa, 2006; Gulima 2009; Tilahun et al., 2012).

A study conducted in the Netherlands from November 2011 to January 2012, showed that there was no significant difference in seroprevalence of SBV between different age groups. This indicated that all age groups to be equally susceptible to a newly introduced virus that entered the country for the first time (Elbers et al., 2012). In the following years, calves born from seropositive cows were shown to have a higher titer of antibodies that was lost 5–6 months later (Elbers et al., 2014). In the current study, more adult cows tested positive for SBV antibodies than younger ones. Perhaps this age-related gradient could be another indication that the virus might have been circulating in Ethiopia well before the first outbreaks in Europe. Higher seroprevalence in adult cows could be the result of the accumulation of exposure over several seasons, the so-called age-cohort effect. In contradiction to ours, other authors have suggested the differences might be attributed to odour attractants from adult cows being more efficient in attracting culicoides than those from young cattle (Méroc et al., 2013).

The final model fit well (Hosmer-Lemeshow χ^2 = 8.88, p-value = 0.1134): but with a limited ROC area of 0.6368. Such lack of strength in the model might arise from the failure of the investigation in capturing some of the significant predictors for SBV dynamics including, vectors, insemination and calving seasons. In this connection, sheep mated before August 2011 and in August 2011 in the Netherlands had increased odds of malformations in newborn lambs compared to sheep flocks with a start of the mating season in October 2011 (Luttikholt et al., 2014) corresponding to the difference in time of vector abundance. Such limitations could be overcome by designing and conducting longitudinal studies which consider both vector dynamics and reproductive practices of cattle in the study areas over time.

Many of the reproductive disorders including abortion, stillbirth, retained fetal membranes, and metritis were unconditionally associated with seroprevalence in a univariable logistic regression analysis. Others such as the birth of weak calves and mean calving intervals and the birth of congenitally defective calves were not. Although some of these clinical manifestations of SBV infection in the current study were also reported in Europe (Garigliany et al., 2012b; Hoffmann et al., 2012), overemphasising the importance of such findings could lead to erroneous conclusions. This could be due to one or more of the following reasons: i) presence of multiple causes of reproductive disorders reported from dairy cattle in Ethiopia; ii) factors such as increased frequency of abortions with increasing seroprevalence of SBV antibodies which appears to contradict with solid immunity developed and absence of the disease in European cattle following 2011–2012 outbreaks (Méroc et al., 2015; Wernike et al., 2015); iii) lack of temporality in exposure to the virus and outcomes of exposure (cross-sectional study) iv) lack of direct empirical evidence for the presence of the virus itself; and v) possibility of cross-reactions with other members of the Simbu serogroup to which SBV belongs. Further studies are recommended to confirm the presence of the virus and its impact on the reproductive performance of cattle in Ethiopia.

The intra-herd correlation coefficient of 0.13 is relatively small,

Table 4
Univariable logistic regression analyses of SBV serostatus in relation to reproductive disorders in dairy cows in central, southern and western Ethiopia.

"Effect"	Categories	No. examined	Prev. (95% CI)	Odds Ratio	p-value
Abortion	No	882	53.5 (50.2–56.8)	Reference	–
	Yes	268	72.0 (66.3–77.1)	2.1 (1.5–2.9)	< 0.001
Abortion frequency	No	882	53.5 (50.2–56.8)	Reference	–
	Single	217	68.7 (62.2–74.5)	1.8 (1.3–2.6)	0.002
	Multiple	51	86.3 (73.7–93.4)	5.1 (2.2–11.8)	< 0.001
Stillbirth	No	1103	57.6 (54.6–60.5)	Reference	–
	Yes	47	63.2 (48.9–75.6)	1.9 (0.98–3.7)	0.056
Birth of weak calf	No	1136	57.7 (54.7–60.5)	Reference	–
	Yes	14	71.4 (42.8–89.3)	1.5 (0.5–4.9)	0.510
Birth of defective calf	No	1144	57.9 (54.9–60.7)		
	Yes	9	55.6 (23.6–83.5)	1.1(0.3–4.5)	0.924
RFM	No	758	54.5 (50.9–58.0)	Reference	–
	Yes	338	65.1 (59.8–70.0)	1.5 (1.1–2.0)	0.009
Metritis	No	742	55.0 (51.4–58.5)	Reference	–
	Yes	358	63.7 (58.6–68.5)	1.4 (1.03–1.8)	0.032
MCI	Expected	314	57.3 (51.8–62.9)	Reference	
	Prolonged	291	65.6 (59.9–70.9)	1.3 (0.89–1.9)	0.175

RFM = Retained Fetal Membranes; Prev. = Prevalence, MCI = Mean Calving Interval, CI = Confidence Interval

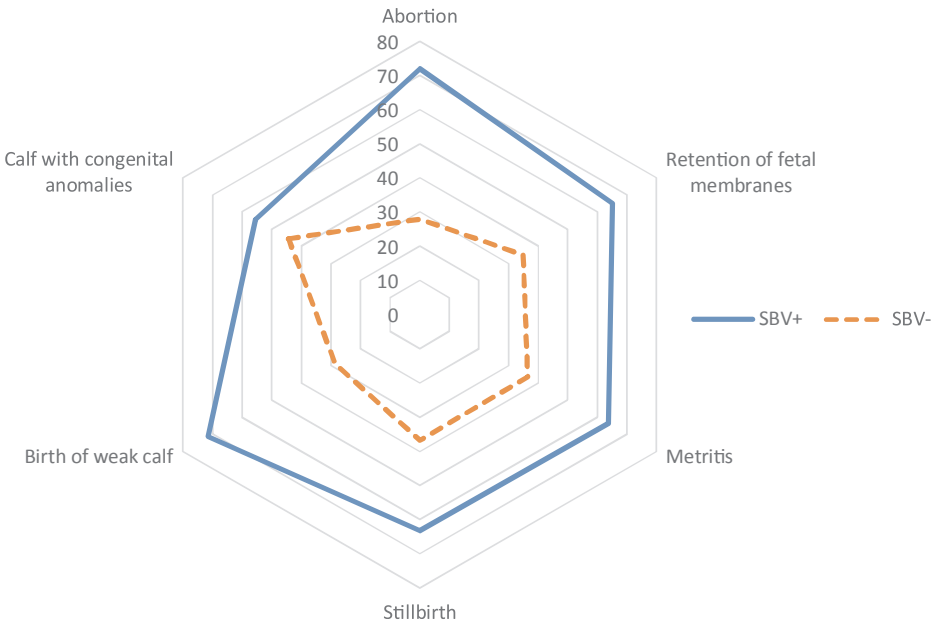

Fig. 2. Proportion of reproductive disorders among SBV seropositive and negative dairy cows in central, southern and western Ethiopia. Higher proportion of dairy cows with exposure to SBV had one or the other type of reproductive disorders than those without.

implying that dairy animals within a herd have a limited in-herd correlation concerning their SBV serostatus. From the design effect of 2.04 computed for this study, it is possible to conclude that the sample size used in the current study is larger than the 745 samples needed to estimate SBV seroprevalence. Thus, the prevalence estimate given is precise enough to ensure internal validity for dairy cattle in the study area. As previously described, one of the limitations of this study was that samples used in the current study were collected without considering risk factors of SBV exposure. The risk factors collected at animal and herd levels, therefore, are not exhaustive and cautious interpretation of the final model is warranted for the virus exposure under study.

5. Conclusions

The prevalence of anti-SBV antibodies in Ethiopia was high (56.6%). It was widely distributed among all the sampled conurbations and a significant majority of herds in central, southern and western parts of the country. It was affected by agroecology, the age of dairy animals and type of farm. Some of the reproductive disorders were found to be associated with seroprevalence of SBV. To the best of the authors' knowledge, this is the first report on SBV seroprevalence in cattle in Ethiopia. Further, well-designed studies on identification of the virus and its impact on livestock in Ethiopia are recommended.

Conflict of interest

None.

Author's contribution

BS and KA designed the study, did part of the fieldwork, and drafted the manuscript. KA analysed the data. GA did the laboratory works and participated in the write-up. EZ took part in field works and participated in the write-up. ES and KA supervised the study, assisted data analysis and interpretation and enriched the manuscript. All authors have read and approved the manuscript.

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