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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

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