Preventive Veterinary Medicine 150 (2018) 126–132

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Bovine herpesvirus-1 in three major milk sheds of Ethiopia: Serostatus and association with reproductive disorders in dairy cattle

Berhanu Sibhat[a,*], Gelagay Ayelet[b], Eystein Skjerve[c], Endrias Z. Gebremedhin[d], Kassahun Asmare[e]

[a] College of Veterinary Medicine, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia
[b] National Veterinary Institute, P.O. Box 19, Debre Zeit, Ethiopia
[c] Norwegian University of Life Sciences, P.O. Box 8146 Dep., N-0033 Oslo, Norway
[d] College of Agriculture and Veterinary Sciences, Ambo University, P.O. Box 19, Ambo, Ethiopia
[e] School of Veterinary Medicine, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

ARTICLE INFO

Keywords:
Abortion
BHV-1
B-ELISA
Dairy cattle
Risk factors
Seroprevalence
Stillbirth
Ethiopia

ABSTRACT

Bovine herpesvirus-1 (BHV-1) causes infectious bovine rhinotracheitis (IBR), and infectious pustular vulvovaginitis (IPV) in cows and infectious pustular balanopostitis (IPB) in bulls worldwide. Infection of seronegative cattle with BHV-1 leads to abortion, retention of fetal membranes, increased service per conception, metritis and oophoritis. As part of an ongoing study on infectious causes of reproductive disorders in Ethiopia, this investigation aims at assessing the role of BHV-1 in the disorders and the risk factors affecting its seroprevalence. A cross-sectional study was conducted on a total of 1379 randomly selected dairy cattle from 149 herds. These dairy cattle were sampled from milks sheds of central (n = 555), western (n = 195) and southern (n = 629) Ethiopia. Blocking enzyme-linked immunosorbent assay (B-ELISA) was applied to detect antibodies specific to BHV-1. Additionally, a semi-structured questionnaire was administered and farm records were assessed to capture potential risk factors associated with BHV-1 seropositivity. Univariable and multivariable random-effects logistic regression analyses were used to assess potential risk factors associated with BHV-1 serostatus. Model fitness and reliability were assessed using the Hosmer and Lemeshow method and the receiver operating curve (ROC) respectively. An overall herd level BHV-1 seroprevalence of 81.8% (95% confidence interval (CI): 74.7–87.7%) and individual animal level seroprevalence of 41.0% (95% CI: 38.4–43.7%) were found. In a random-effects multivariable logistic regression model, the seroprevalence of BHV-1 exposure was higher in dairy cattle from breeding (Odds ratio [OR] = 1.3; p = 0.036) than in commercial (OR = 0.9; p = 0.137) and small-holder farms. Geographically, the prevalence was higher in western (OR = 1.4; p < 0.001) and southern Ethiopia (OR = 1.2; p < 0.001) than in central regions. BHV-1 seropositive cows had higher (p < 0.05) odds of clinical reproductive disorders including abortion, retained fetal membranes, stillbirth, birth of weak calf and metritis compared to seronegative cows. Thus, it is suggested that BHV-1 should be considered as differential diagnosis among improved dairy cattle herds with reproductive disorders in Ethiopia.

1. Introduction

Bovine herpesvirus-1 (BHV-1) causes important cattle diseases such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB), in cows and bulls worldwide. The virus belongs to genus *Varicellovirus in* the subfamily *Alphaherpesvirinae* of the family *Herpesviridae* (Muylkens et al., 2007). BHV-1 has three serologically indistinguishable strains (BHV-1.1, BHV-1.2a and BHV-1.2b). Although not absolute, under natural conditions, BHV-1.1 is mainly isolated from cases of IBR while BHV-1.1 and 1.2a were frequently isolated from aborted fetuses associated with IBR. Strain 1.2b however, is associated with IPV in cows and IPB in breeding bulls but not with cases of abortion (Graham, 2013).

Abortion occurs as a consequence of infection of seronegative cows by BHV-1. The virus reaches the fetus by crossing the placental-blood-barrier following systemic spread through viremia in animals afflicted by IBR (Muylkens et al., 2007). It causes the death of the fetus before degeneration of the placenta occurs, hence there is a delayed expulsion

* Corresponding author.
E-mail addresses: berhanusibhat@gmail.com (B. Sibhat), gelagayayelet1@gmail.com (G. Ayelet), eystein.skjerve@nmbu.no (E. Skjerve), endrias.zewdu@gmail.com (E.Z. Gebremedhin), ka7588@yahoo.com (K. Asmare).

https://doi.org/10.1016/j.prevetmed.2017.12.019
Received 27 May 2017; Received in revised form 25 December 2017; Accepted 31 December 2017

resulting in in-utero autolysis of the fetus (Molello et al., 1966; Kendrick and Straub, 1967) and frequent retention of fetal membranes. Abortions typically occur during 4–8 months of gestation within 15–64 days post infection regardless of the stage of pregnancy (Gibbs and Rweyemamu, 1977). Other reproductive disorders associated with BHV-1 include infertility with increased service per conception, metritis, and oophoritis (Graham, 2013). Another important epidemiological feature of BHV-1 is that infections usually produce latency. Such cases may be reactivated later on following exposure to stressors (OIE, 2010). It has been suggested that late abortions occurring up to 100 days post infection might be the result of such reactivations (Jones and Chowdhury, 2008).

The virus is distributed worldwide, except the BHV-1-free countries (OIE, 2010). Studies conducted in different countries across the world over the last 15 years reported varying seroprevalence ranging from 35.9–77.5% in Europe and 37–60.8% in Latin America (Raaperi et al., 2014). Recent reports from Sub-Saharan Africa on BHV-1 seroprevalence in cattle are scarce, but include 48.3% in Southern Zambia (Mweene et al., 2003), 69% in Ghana (Adu-Addai et al., 2012) and 74.5% in Gauteng province of South Africa (Njiro et al., 2011).

In Ethiopia, two preliminary surveys conducted in limited geographic areas in the mid-1970s and late 1980s demonstrated serological evidence of the presence of the virus in the country. Accordingly, the seroprevalence of BHV-1 was 41.8% in Harar and Sidamo provinces (Lefevre, 1975) and 67% in Gobe and Ghibe in central Ethiopia (Bekele et al., 1989). Apart from the serological evidence in the two reports, the importance of the virus as the cause of reproductive disorder in cattle in Ethiopia has never been investigated.

As part of an ongoing project that started in 2012 on identification of possible infectious causes of reproductive disorders of dairy cattle, this study was conducted to estimate the seroprevalence of BHV-1 and identifying factors affecting its occurrence in southern, central and western part of Ethiopia where dairying is practiced using improved dairy cattle breeds. The study also assessed the potential role of BHV-1 as cause of specific reproduction disorders in these group of cattle.

2. Materials and methods

2.1. Study area

The study was conducted in 15 conurbations in central, southern and southwestern parts of Ethiopia including the capital Addis Ababa. The areas were selected based on relative abundance of dairy farms and long tradition of keeping improved dairy cattle. The selected conurbations represent well-known milk sheds supplying milk to major urban and peri-urban areas of the country. Central milk shed refers to dairy farms located in central part of the country, i.e. Addis Ababa, Adama, Ambo, Sebeta, Holeta, Bishoftu and Adda Berga. The southern milk shed on the other hand includes Arsi Negele, Allage, Shashemene, Hawassa, Wendo Genet, Hossaena and Wolaita Sodo. In the west Jimma and its surroundings. The central and southern milk sheds are the oldest in the country and serve as sources of dairy animals for the establishment of new dairy farms as well as for expansion of existing farms in other parts of the country (Asmare et al., 2013).

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 in and on the outskirts of major towns located in the study areas. The study also includes four government owned breeding farms. Small-holder dairy farms are those holdings with up to 10 dairy cattle. These farms produce milk for household consumption and commercial purposes. While commercial dairy farms had more than ten dairy cows and produce milk basically for sale. Three of the breeding farms are established by the government for dairy improvement through crossbreeding of high blood European cattle breeds (HF, Jersey) with local zebu cattle with the objectives of distributing, primarily, pregnant crossbred heifers to rural small-holder dairy farmers. One breeding farm located at Holeta is a bull rearing facility keeping Holstein-Friesian cows for raising male calves that would later become bulls based on performance of their dams for the National Artificial Insemination Center, a facility that produces and distributes semen for artificial insemination services throughout the country.

Cattle breeding in Ethiopia is done either with artificial insemination, natural bull mating or both depending on the availability of the former or farmer's preference. Similarly, calving occurs throughout the year.

2.3. Study design

This cross-sectional study involved analysis of blood samples from randomly sampled dairy cattle and individual and farm level data acquired from farm records where available and interviews using a semi-structured questionnaire. As most commercial, urban and peri-urban small-holder dairy farms are situated in and around major towns, 15 major conurbations were purposively selected considering the density of dairy farms in and around them. In collaboration with the respective district veterinary departments, the sampling frame of dairy farms was prepared. In each conurbation, a minimum of 10% of the herds or farms was randomly selected. In each farm a minimum of 10% of female animals above six months of age were randomly selected. For breeding cattle, the sampling design followed similar pattern except for the herd level selection which was purposive. Altogether four breeding, 125 small scale and 20 commercial dairy herds were selected from western, southern and central Ethiopia.

2.4. Sampling and sample size

The minimum sample size was determined based on a 67% individual animal level prevalence (Bekele et al., 1989), 95% level of confidence, and 5% absolute precision (Thrusfield, 2007), requiring a minimum sample size of 339 animals. The sample size was proportionally allocated to the three regions based on the 2011 livestock survey results (CSA, 2011). Accordingly, 50% of the samples (n = 169) were assigned to central followed by 40% (n = 136) to south and 10% (n = 34) to western Ethiopia. However, considering the design effect, the calculated sample size was expanded by 4 times to account for intra-herd variability and a total of 1379 animals were sampled from 149 herds, out of which 555, 629, 195 were from central, southern and western Ethiopia, respectively.

2.5. Blood samples and serological assay

Blood samples were collected from jugular vein or coccygeal artery using plain vacutainer tubes and disposable needles. From each randomly selected animal, 10 ml of blood was drawn and kept overnight in an upright position followed by centrifugation at 1000 * *g* for 10 min. The serum samples were decanted into sterile cryovials, labeled and transported on ice to National Veterinary Institute at Bishoftu (Debre Zeit) where they were preserved at −20 °C until screened. A Blocking immunoenzymatic assay (B-ELISA) with sensitivity of 93% and specificity of 99% was applied to detect antibodies specific for BHV-1 as per the manufacturer's protocol (INgezim IBR Compac, Ingenasa, Spain). Briefly, 50 µl diluent (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 50 µl sera (test samples, negative and positive control) were added according to the plate layout into BHV-1 antigen pre-coated plate. The plate was incubated for an hour at 37 °C and washed five times. 100 µl of the conjugate was added to all wells, incubated at 37 °C for 30 min and washed out. Finally, 100 µl of substrate solution was added, kept at room temperature in a dark place for 15 min, stopped by 100 µl stop