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Association between antibody status to bovine herpesvirus 1 and quality of milk in dairy herds in Poland

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ABSTRACT

Bovine herpesvirus 1 (BoHV1) is one of the most important pathogens of cattle; however, its effect on somatic cell count and milk components is not completely understood. The aim of the current study was to examine the effect of BoHV1 infection on quality of bovine bulk tank milk (BTM). A total of 1,790 individual blood samples collected at 28 dairy farms were used to determine the BoHV1 infection status of the herds with ELISA tests. The quality parameters of milk were evaluated by instrumental methods with BTM samples collected at monthly intervals from May 2011 to May 2012. The statistical analysis was performed to study the associations between BoHV1 herd status, quality of BTM, and herd-specific parameters. The risk factors influencing bulk milk somatic cell count (BMSCC) were estimated using the multivariable mixed-effects maximum likelihood regression model. The true prevalences of BoHV1 infection at the animal and herd levels were 49.3 and 64.6%, respectively. The average BMSCC differed significantly between the herds grouped accordingly to their BoHV1 infection status. Interestingly, the highest BMSCC was observed in the vaccinated herds (240.3×10^3 cells/mL). Additionally, the BoHV1 herd status had a significant effect on the fat content of BTM. The largest herds that were investigated had a BoHV1 seroprevalence over 30%. The herd status was considerably influenced by the numbers of cows in the herds. Besides, no significant differences in total bacterial count or protein content in milk from BoHV1-infected und uninfected herds were observed. An increase in BMSCC was observed during summer compared with the winter months regardless of the BoHV1 status of the herds. In the final multivariable regression model, the main risk factors associated with BMSCC were BoHV1 herd status, the percentage of BoHV1 infected animals in a herd, the number of cows in a herd, and the season. Our study suggests that BoHV1 infection may influence BMSCC levels, which are key parameters of BTM quality and a reference for subclinical mastitis in a herd. In conclusion, BoHV1 infection may cause economic losses by decreasing both quantity and quality of milk.
Key words: bovine herpesvirus 1, bulk tank milk, somatic cell count

INTRODUCTION

Bovine mastitis is one of the most economically important diseases affecting the dairy cattle sector all over the world. It is characterized by physical, chemical, and, usually, microbiological changes in the milk and pathological lesions in the glandular tissue of the udder (Sharma, 2007). Subclinical mastitis is still the most prevalent form of mastitis causing the greatest economic losses in spite of intensive research and implementation of various control programs (Pyörälä, 2003). Cows with subclinical mastitis are difficult to detect due to the absence of visible symptoms of infection. Currently, according to the recommendations of the International Dairy Federation (1999), subclinical mastitis is diagnosed based on milk SCC.

The primary agent of mastitis is a wide spectrum of bacterial strains; however, incidences of viral-, mycoplasmal-, algal-, and fungal-related mastitis have also been reported (Barkema et al., 2009). Bovine herpesvirus 1 (**BoHV1**), responsible for infectious bovine rhinotracheitis (**IBR**), infectious pustular vulvovaginitis in cows, and infectious pustular balanoposthitis in bulls, is presumably associated with mastitis (Wellenberg et al., 2002). Following an acute infection, BoHV1 establishes a latent infection, and after reactivation caused by stress factors it is re-excreted and can infect susceptible animals. Therefore, once infected, animals must be regarded as lifelong potential shedders of the virus (Pastoret et al., 1982). Bovine herpesvirus 1 causes significant economic losses, mainly attributable to reduced milk production, reproductive failure, abortions, increased calf mortality, and restrictions in

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the international trade of livestock (Nandi et al., 2009). The virus has the ability to induce immunosuppression by impairing the cellular immune response, which may theoretically increase the risk of mastitis as well (Jones and Chowdhury, 2010). Although BoHV1 infections are globally distributed, significant differences in regional incidences and prevalences were found. The average seroprevalences of BoHV1 infection in the United States, Canada, Australia, and New Zealand are very high (Ackermann and Engels, 2006). The European countries of Austria, Denmark, Finland, Norway, Sweden, and Switzerland are officially free of IBR. The seroprevalences of BoHV1 in other European countries range from 14% in Lithuania (Jacevičius et al., 2008) to 83% in southwest England (Woodbine et al., 2009). Eradication of IBR is often based on vaccination with glycoprotein E (**gE**)-deleted marker vaccines. In Poland, 37.7% of dairy cows and 73.7% of dairy cattle herds were positive for BoHV1 antibodies (Rola et al., 2005). In Poland, bulls at the national semen-collection centers are free from BoHV1 infections; however, no official IBR-eradication program was established in dairy herds. The aim of the current study was to examine the effects of BoHV1 infection on quality of milk produced in dairy herds in eastern Poland.

MATERIALS AND METHODS

Dairy Herds

Farms with more than 10 cows, controlled for milk performance and located in eastern Poland, were selected for the present study. Twenty-eight out of 40 farmers who received information concerning our study agreed to participate. All of the farms supplied milk to the Spomlek Dairy Cooperative (Radzyn Podlaski, Poland). The size of dairy farms ranged from 24 to 186 (average 64) milking cows of the Holstein-Friesian breed. Cows were managed in open-stabling systems and fed using TMR technology. Eight of the 28 herds were vaccinated with a BoHV1 gE-deleted marker vaccine (Rispoval IBR-Marker Inactivatum, Pfizer, Louvain-la-Neuve, Belgium). During the observation period, the farms were bound to follow the biosecurity measures that included quarantine and testing for BoHV1 of newly purchased cattle, limited access of visitors, and protective clothing for farm staff.

BoHV1 Herd Status

To determine the BoHV1 infection status of the farms, 1,790 individual blood samples from milking cows were collected in May 2011. Additionally, bulk tank milk (**BTM**) samples from all herds were collected 3 times during the period from May 2011 to May 2012. All samples were submitted to the laboratory within 2 working days after collection. Blood samples were centrifuged (3,000 × *g* for 15 min at 4°C) and serum was collected and frozen at −20°C until testing, whereas BTM samples were kept at 4°C not longer than 3 d. In unvaccinated herds, serum samples were tested with the commercial Idexx IBR glycoprotein B (**gB**)-blocking ELISA test kit (Idexx, Montpellier, France) with a specificity of 99.8 (95% CI: 99.4–100%). Briefly, 100 μL of 1:2 diluted serum samples in dilution buffer serum samples, positive and negative control sera, were added to BoHV1-coated microplates and incubated for 2 h at 37°C. Then, the microplates were washed 3 times with wash solution and 100 μL of conjugate diluted 1:100 was added. After incubation (30 min, 37°C) the microplates were washed again and tetramethylbenzidine (TMB) substrate was added. After 10 min of incubation at room temperature, the reaction was stopped by adding stop solution and the optical density was measured at 450 nm. A sample was classified as positive when the sample/mean negative controls (**S/N**) ratio was less or equal to 50%. In vaccinated herds, the Idexx IBR gE ELISA test kit with a sensitivity of 99.83 (95%CI: 99.7–100%) was used. A sample was defined as positive for antibodies to the gE glycoprotein of BoHV1 when the S/N ratio was less or equal to 0.6. A herd was considered to be infected with BoHV1 if at least 1 serum sample was positive in the respective ELISA test. Antibodies to BoHV1 in BTM samples were detected with the Idexx IBR gE ELISA test kit. Milk sample was classified as positive for antibodies to the gE glycoprotein when the S/N ratio was less or equal to 0.8. All tests were performed according to the manufacturer's instructions. The BoHV1 herd status was established by testing of individual sera, and the herds were consequently divided into 4 groups (Table 1).

BTM Quality Parameters

Analyses of bulk tank milk somatic cell count (**BMSCC**), total bacteria count (**TBC**), and fat and protein contents were performed based on the reports issued by the Resources Assessment Laboratory. All applied methods were validated and the qualifications of laboratory staff were confirmed by the Polish Centre for Accreditation (Warsaw, Poland). The BMSCC was determined by flow cytometry using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark), TBC by flow cytometry with a BactoScan FC (Foss Electric), and fat and protein content by infrared spectrometry with a MilkoScan 6000 (Foss Electric) according to the manufacturer's instructions. The BTM samples from