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Short communication

Bulk tank milk somatic cell counts in dairy herds with different bovine viral diarrhoea virus status in Poland

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ABSTRACT

The aim of the study was to examine the effect of bovine viral diarrhoea virus (BVDV) infection on bulk tank milk somatic cell counts (BMSCC). Twenty nine dairy farms supplying milk to a dairy in Eastern Poland were recruited for the study. Bulk milk ELISA and RT-PCR were used to determine the BVDV infection status and the presence of PI animals in the farms. The BMSCC mean values for the BVDV seronegative (218.7×10^3 cells/ml; SD: 89.8) and seropositive (214.9×10^3 cells/ml; SD: 74.0) herds did not differ significantly. To assess the relationship between BVDV infection and BMSCC a multilevel mixed-effects linear model was used. No statistically significant effect of BVDV infection on BMSCC was found. The mean values of BMSCC for the herds with PI individuals measured before (230.1×10^3 cells/ml, SD: 64.9) and after (223.3×10^3 cells/ml, SD: 62.4) the PI removal were not statistically different. An increase in herd size was associated with a significant decrease in BMSCC. An increase in BMSCC was observed during summer (from May to September) compared to during winter (from October to April).

1. Introduction

Bovine mastitis is one of the most economically important production diseases affecting the dairy cattle sector all over the world. It is characterized by physical, chemical and usually bacteriological changes in the milk and lesions in the glandular tissue of the udder (Sharma, 2007). In two studies from 2005, mastitis infections were estimated to cost the EU dairy industry about €1.55 billion (Hillerton and Berry, 2005) and the US industry $2 billion annually (Losinger, 2005). Subclinical infections result in a decreased milk yield, deterioration of milk quality and increased risk of culling of chronically infected animals (Huijps et al., 2008). 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 subclinical mastitis is diagnosed based on milk somatic cell count (SCC).

Microorganisms causing mastitis have been divided into major and minor pathogens depending on the severity of inflammatory response to the infection. Minor pathogens are most often associated with subclinical mastitis and they include microorganisms like coagulase negative staphylococci, mycoplasms, fungi, yeasts, chlamydia and viruses (Barkema et al., 2009; Wellenberg et al., 2002). Bovine viral diarrhoea virus (BVDV), responsible for multi-organ infection of the host, is also regarded to be associated with udder health problems in dairy herds (Berends et al., 2008). BVDV

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infections occur in many countries and they cause significant economic losses, mainly attributable to reduced milk production, reduced reproductive performance, delayed growth, increased susceptibility to other diseases and high mortality among young stock (Houe, 1999). BVDV has the ability to induce immunosuppression by impairing the cellular immune response (Potgeiter, 1988) which may theoretically increase the risk of mastitis as well. The herd-level seroprevalence of BVDV in the EU countries range from below 1% in Finland to 95% in England (Greiser-Wilke et al., 2003). In Poland, BVDV herd prevalence is over 70% when measured in bulk tank milk (BTM) samples (Kuta et al., 2013). In a previous Polish study the percentage of infected herds ranged from 53.9% to 100% depending on herd size (Rypuła et al., 2010). However, there is no accurate data on the prevalence of persistently infected (PI) animals in Polish dairy cattle population, although the results of a recent study indicate that the number of such animals does not exceed 2.5% (Kuta et al., 2013).

The aim of the study was to examine the effect of BVDV infection on the somatic cell counts in bulk tank milk produced in dairy herds from Eastern Poland.

2. Materials and methods

2.1. Dairy farms

Farms with more than 10 cows, controlled for milk performance and located in Eastern Poland were selected for the study. Twenty nine out of forty farmers who received information concerning this 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 125 milking cows of the Holstein-Friesian breed. Cows were managed in free-stall systems and fed using TMR (total mixed ration) technology. No BVDV vaccination was used in any of the farms. During the observation period the farms were bound to follow the biosecurity measures that included quarantine and testing for BVDV of newly purchased cattle, limited access of visitors and protective clothing for farm staff. After identification of BVDV PI animals in November 2011 they were culled as soon as possible.

2.2. BVDV herd status

To determine BVDV infection status of the farms, 29 bulk tank milk (BTM) and 1663 individual serum samples from milking cows were collected in November 2011. Serum samples were submitted to the laboratory within 2 working days after collection and they were frozen at −20 °C until testing while BTM samples were kept at 4 °C not longer than 3 days. The commercial SVANOVIR BVDV-Ab ELISA kit was used for the detection of antibodies to BVDV in BTM, according to the manufacturer's instruction (SVANOVA, Sweden). The sensitivity and specificity declared by the company were 100% and 98.2%, respectively. The corrected optical density (COD) was calculated for each BTM sample by subtracting the OD value of negative control. The BTM samples below and above the cut off value of 0.25 were considered negative and positive, respectively (first BVDV status predictor). Furthermore, the obtained results were interpreted according to the Swedish classification system by which herds are divided into four different classes based on COD values (second BVDV status predictor) (Niskanen, 1993; Kampa et al., 2004). Herds in classes 0 and 1 (CODs < 0.05 and between 0.05 and 0.24, respectively) have a very low and low level of antibodies in bulk milk and are unlikely to contain PI animals. Herds in classes 2 and 3 (CODs between 0.25 and 0.54 and ≥0.55, respectively) have a moderate and high level of antibodies. The PI animals are usually found in farms belonging to class 3 (Lindberg and Alenius, 1999).

The farms containing PI animals were identified based on the results of RT-PCR of the serum samples. To reduce the costs of testing, the sera from individual farms were pooled into groups of up to 25 samples. In house validation of RT-PCR using pooled samples showed positive signal at dilutions 1/50 to 1/200 of positive serum while bulk tank milk was positive even in a pool from 556 cows with a single PI animal (Kuta et al., 2013). Therefore the sensitivity of RT-PCR in pooled serum samples enabled the identification of a single positive sample pooled at least with 49 samples. Specificity of the test was always above 90% except for the accidental contamination encountered on single occasions and non-specific bands observed in samples from vaccinated animals. If a pool reacted positively, individual samples were tested with RT-PCR to identify PI animals in a herd (two positive results on samples collected at least 3 weeks apart to exclude transient infections). The total RNA was extracted from 500 μl of serum using TRI Reagent (Sigma-Aldrich, USA), according to the manufacturer's instruction and stored at −70 °C until testing. RT-PCR was performed according to the protocol described previously by Vilcek et al. (1994) with the primers flanking 5′ untranslated region of viral genome.

2.3. BTM quality parameters

Analyses of bulk tank milk somatic cell count (BMSCC), total bacteria count (TBC), fat (FAT) and protein (PROT) content were performed based on the reports issued by the Resources Assessment Laboratory. All methods used were validated and the qualifications of laboratory staff were confirmed by the Polish Centre for Accreditation (Warsaw, Poland). BMSCC was determined by Fossomatic 5000, total bacteria count with a Bactoscan FC and fat and protein content with a Milcoscan 6000, according to the manufacturer's instructions. The BTM samples from all herds were tested monthly between May 2011 and May 2012 (13 time points).

2.4. Statistical analysis

The true prevalence of BVDV antibodies and PI animals in the herd were estimated from the apparent prevalence using Rogan and Gladen (1978) correction. A multivariable mixed-effects linear regression using generalized estimation equation (GEE) with BMSCC as the dependent variable was fit using STATA software (StataCorp., College Station, TX, USA). The associations between BMSCC and BVDV herd status (BTM seropositivity, COD value, BVDV herd class,