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Bovine leukemia virus infection in cattle of China: Association with reduced milk production and increased somatic cell score

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ABSTRACT

The main objective of this study was to investigate the individual cow effect of bovine leukemia virus (BLV) infection on milk production and somatic cell score (SCS). The fluorescence resonance energy transfer (FRET) quantitative PCR established in this study and a commercial ELISA kit revealed that 49.1% of dairy cattle (964/1,963) from 6 provinces of China and 1.6% of beef cattle (22/1,390) from 15 provinces were BLV positive. In a detailed study of 105 cows, BLV was found most commonly in buffy coat samples that also had highest copy numbers (104.75 ± 1.56 per mL); all cows negative for BLV in buffy coat samples were also negative in vaginal swab, milk, and fecal samples. Copy numbers of BLV were 102.90 ± 0.42/gram of feces, 100.83 ± 0.62/mL of milk, and 102.18 ± 0.81 per vaginal swab. The BLV-positive cows had significantly lower milk production in the early (26.8 vs. 30.9 kg) and middle stages of lactation (22.2 vs. 26.1 kg) in animals with ≥4 parities than the BLV-negative cows; they also had significantly higher SCS in early and middle lactation stages (early = 5.2 vs. 4.3; middle = 4.9 vs. 3.9) in animals with ≥4 parities. Milk production and SCS did not significantly differ between the BLV-infected and -uninfected cows when they were in the late lactation stage or in animals with ≤3 parities. Taken together, our results indicate that BLV infections are widespread in the dairy farms of China. Vaginal secretions and feces may be involved in BLV transmission. A BLV infection may result in reduced milk yield and increased SCS in a parity and lactation stage-restricted manner.

Key words: bovine leukemia virus, China, milk production, somatic cell score

INTRODUCTION

Enzootic bovine leukosis (**EBL**) is caused by bovine leukemia virus (**BLV**), which belongs to the family of Retroviridae. Whereas most BLV-infected cattle stay asymptomatic as carriers, approximately 30% develop persistent lymphocytosis and 5% die from malignant lymphoma (Schwartz and Lévy, 1994; Bartlett et al., 2013).

Cattle are free of EBL in many European countries (Nuotio et al., 2003; Acaite et al., 2007; European Commission, 2014), and Australia and New Zealand have nearly succeeded in eradicating this disease (Farm Biosecurity, 2011; Kobayashi et al., 2014). In many other regions of the world, however, the disease is widespread. For example, in the maritime region of Canada, the herd-level prevalence of BLV was 90.8% based on bulk tank milk census (Nekouei et al., 2015) and 84% of dairy herds in the United States were reported positive in 2007 (USDA, 2008). In South America, the individual prevalence rate in dairy cows is reported to be approximately 33 to 50%, with herd-level prevalence in dairy cows over 84% (Trono et al., 2001). In East Asia, 79% of dairy farms in Japan were found to have BLV seropositive cattle (Kobayashi et al., 2010).

In China, BLV infection in cows was first reported in a dairy herd of Urumqi of Xinjiang autonomous region in 1978 (Deng et al., 1978). Since then, several studies have described BLV infections in Jiangsu, Jilin, Jiangxi, Hubei, Hunan, Shanghai, and Chongqing of China (Dong et al., 1981; Du and Li, 1982; Chen et al., 1983, 1988; Yang et al., 2010; Li et al., 2011). These publications, however, are all in local Chinese journals and a search of the PubMed database with key words "bovine leukemia virus" and "China" (accessed on December 22, 2015) revealed no publications in peer-reviewed international journals regarding BLV in China. Lack of reliable epidemiological data on BLV prevalence in China has led to low awareness of the disease among local and international animal health workers and dairy producers.

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Studies in the United States (Ott et al., 2003) and Canada (Sargeant et al., 1997) have demonstrated a negative effect of BLV infection on milk production at the herd level. Similarly, Olson (1974), Da et al. (1993), and D′Angelino et al. (1998) showed that animals with persistent lymphocytosis and lymphosarcoma caused by BLV infection had reduced milk yields. However, no association has been found between BLV infection and milk production in several studies in individual animals (Jacobs et al., 1991; Kale et al., 2007; Tiwari et al., 2007). Many factors influence milk production and SCS, but few studies have been carried out on how factors such as breed, parity, and lactation stage might have an influence in a multivariate analyses. Although BLV has been reported to affect cells of both the innate and adaptive immune system and alter proper functioning of uninfected cells (Frie and Coussens, 2015) and in the mammary tissue of cows with subclinical mastitis (Yoshikawa et al., 1997), the significance of its presence in the udder and its role in mastitis is unknown. Whereas horizontal transmission of infected blood is considered to be the major route of BLV transfer, BLV detection in milk has been used as the main approach to determine the BLV prevalence in dairy farms (Kobayashi et al., 2010). It would be interesting to compare the BLV copy number in blood, milk, vagina, and feces for a better understanding of the BLV prevalence and transmission. Dairy cattle from 19 provinces of China were investigated in our study to improve the available knowledge of BLV in China and the individual cow effect of BLV infection on milk production and SCS.

MATERIALS AND METHODS

Ethics Statement

Protocols for the collection of samples in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Yangzhou University College of Veterinary Medicine.

Blood Samples from Dairy and Beef Cattle

Convenience whole-blood samples from apparently healthy cattle in 19 provinces of China submitted to the China Animal Health and Epidemiology Center (Shandong, China) for an epidemiological survey of brucellosis between March 2013 and November 2014 were used for this study (Table 1). The 4-mL samples were collected into EDTA and transported at room temperature to the laboratory where buffy coats and plasma were separated and stored at −80°C until being thawed at room temperature for DNA extraction, PCR, and ELISA, as described below.

Yangzhou Dairy Farm

To establish the distribution of BLV in infected animals, convenience whole-blood, milk, vaginal swab, and fecal samples were collected from cows on a dairy farm in Yangzhou of Jiangsu province found to have a high BLV prevalence. Whole-blood samples were collected as described, whereas feces (around 1 g) were collected from the rectum into sterile 1.5-mL tubes and cytobrush vaginal swabs were collected into sterile tubes containing 400 μL of DNA/RNA stabilization buffer (Roche Molecular Biochemicals, Indianapolis, IN). The milk samples (around 10 mL) were collected into sterile tubes after the teats had been wiped with 70% ethyl alcohol and the first milk fractions obtained by hand-milking were discarded.

All samples were transported on ice to the Yangzhou University College of Veterinary Medicine, where aliquots of the whole-blood samples were used for buffy coat collection and plasma and the remainder for routine complete blood counts (BC-2800 Vet, Mindray, Shenzen, China) and biochemical profiles (Vet Test 8008, Idexx Laboratories, Westbrook, ME; Tables 2 and 3).

Shanghai Dairy Farm

A Shanghai dairy found to have a high prevalence of BLV was visited 2 wk later by research investigators to survey the husbandry practices and the environment. Further, whole-blood samples were collected at the beginning of this study and a year later for PCR detection of BLV, but only those cows positive or negative for BLV at both time points were included in the data analysis. The lactation stage (early stage = 1–100 DIM; middle stage = 101–200 DIM; late stage = 201–305 DIM), parity, and BLV status of the cows were recorded (Table 4, Table 5) and milk samples were collected on the fourth day of every month to determine SCC (Shanghai DHI Test Center, Shanghai, China) for SCS calculation as described by Shook and Schutz (1994).

Serological Assay

The Ingezim BLV Compac 2.0 blocking ELISA kit (Ingenasa, Madrid, Spain) was used to detect antibodies in the plasma against BLV gp51 protein according to the manufacturer's instructions.

DNA Extraction from Buffy Coats, Vaginal Swabs, Milk, and Feces

Buffy coats (200 μL) of whole-blood samples were used for DNA extraction with Roche High Pure PCR