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Using PCR for early diagnosis of bovine leukemia virus infection in some native cattle

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ABSTRACT. Bovine leukemia virus (BLV), the causative agent of enzootic bovine leukosis, is an exogenous, B lymphotropic retrovirus belonging to the Retroviridae family that induces persistent lymphocytosis in cattle and sheep. PCR has proven to be particularly suitable for investigating herds of cattle with a very low incidence of BLV infection and for clarifying doubtful serological results obtained by immunodiffusion or ELISA. The native Iranian and Russian cattle have a series of valuable traits that discriminate them as unique breeds that are well able to compete with western analogues. However, their gene pools have not been analyzed with molecular markers, including detection of BLV by PCR. Two pairs of primers were used: gag1 and gag2, and pol1 and pol2, which encompass 347- and 599-bp fragments of the BLV gene, respectively. Sixty-five Iranian Sistani, 120 Yaroslavl, 50 Mongolian, and 35 Black Pied cows were investigated. Among these 270 animals, we obtained 42 positive and 15 doubtful results in the first PCR. The second PCR was very effective in increasing BLV test reliability data to support detection of BLV.

Key words: Bovine leukemia virus; Leukosis; Cattle; PCR; BLV testing; Native Iranian and Russian breeds

INTRODUCTION

Bovine leukemia virus (BLV), the causative agent of enzootic bovine leukosis, is an exogenous, B lymphotropic retrovirus, belonging to the family Retroviridae (Figure 1), which induces persistent lymphocytosis in cattle and sheep. The virus is mainly transmitted horizontally by direct exposure to biological fluids (blood, milk, saliva, and semen) contaminated with infected lymphocytes. After infection, the BLV elicits in immunologically mature cattle a strong immune response against the envelope protein gp51. These antibodies can be detected by ID and also by ELISA tests. Several authors have shown that it is possible to establish BLV-free herds by identifying seropositive animals and eliminating them from the herds (Hoff-Jorgensen, 1989; Klintevall et al., 1991; Lorin et al., 2007). Others demonstrated that serological control is not sensitive enough to find all BLV-infected cattle (Jacobs et al., 1992; Eaves et al., 1994; Ramanavicius et al., 2007). Problems also arise with animals exhibiting periodically or permanently low titers of BLV antibodies or low serum titers in the periparturient period (Coulston et al., 1990; Stone et al., 2000). Furthermore, serological tests cannot discriminate between passive maternal immunity and active immunity induced by bovine leukemia infection (Agresti et al., 1993; Eaves et al., 1994; Beier et al., 2004). Analyzing animals over a long period, Eaves et al. (1994) showed that some naturally infected BLV provirus-carrying animals developed no BLV antibody titers detectable by ID or ELISA for several months or years after infection. In infected cells, BLV is integrated into host DNA in the form of a provirus, which can be detected by different molecular biology methods. Recently, polymerase chain reaction (PCR) for the detection of BLV proviral DNA has been described (Naif et al., 1992; Klintevall et al., 1994; Marsolais et al., 1994; Menendez-Arias, 2002; Czarnik et al., 2002; Amills et al., 2004). Due to some advantages of the PCR method over serological tests, many research groups developed this enzymatic reaction for BLV detection. Several authors have also shown that PCR is particularly suitable for controlling herds of cattle with a very low incidence of BLV infection and for clarifying doubtful serological results obtained by ID or ELISA.

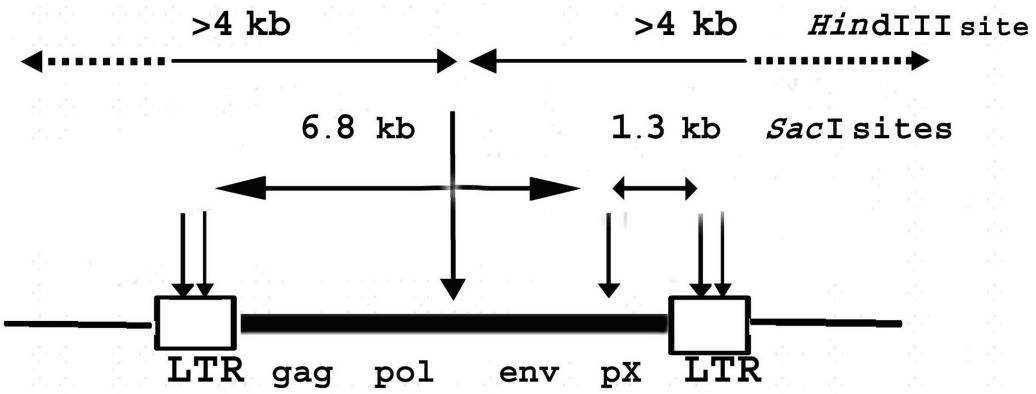

Figure 1. Structure of bovine leukemia virus (BLV) provirus. The predicted sizes of fragments and the sites of cleavage by *Sac*I and *Hin*dIII are also shown. *Sac*I can generate two major fragments (6.8 and 1.3 kb), while *Hin*dIII can generate two fragments of more than 4.0 kb in length per integrated copy of the complete BLV provirus.

In Russia and Iran, despite adopted efforts, the prevention and eradication of viral infections in farm animals have always been a problem. The latent infection with retroviruses such as BLV complicates early diagnosis of disease caused by the viruses. The long-term (for 3-4 years) observations of cattle-breeding farms revealed that eradication of bovine enzootic leukosis in these two countries by the available traditional preventive and sanitary methods is impossible (Licursi et al., 2003; Snider et al., 2003). Native Iranian and Russian breeds have a series of valuable traits that discriminate them as unique breeds that are well able to compete with Western analogues. On the other hand, their gene pools, to date, have not been analyzed with molecular markers, particularly in regard to BLV detection by PCR. The aim of this research was to detect proviral BLV DNA in Iranian Sistani, Russian Yaroslavl, Mongolian, and Black Pied cattle by PCR and compare our results with the data previously obtained in samples of other breeds of cattle.

MATERIAL AND METHODS

A total of 65 Iranian Sistani, 120 Yaroslavl, 50 Mongolian, and 35 Black Pied cows were investigated. Blood samples of 6 mL were collected from all animals in good health by venipuncture. As an anticoagulant, Wersen II (8.0 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 50.0 g EDTA in 1000 mL H_2O) was used at 1:20 dilution. A 200-µL aliquot of the blood was used and DNA was isolated by the Diatom™ DNA Prep 200 kit (IsoGene, Moscow). The total DNA amount and its concentration were estimated by comparison with phage DNA lambda and agarose/ethidium bromide gel electrophoresis. Two pairs of primers: gag1: 5'GGAGGTGGGAAGATGCGAACTATT3' and gag2: 5'GTCCGCTCTACCAACCCTGA ACTT3', as well as pol1: 5'GAGGTTTGTGCATGACCTACGAGCTACA3' and pol2: 5' TAGAG ACCCATTGGAGGTCTCCTAAGAC3', which encompassed 347- and 599-bp fragments of the BLV gene, respectively, were used. PCR was performed in a total reaction volume of 25 µL and about 100-150 ng DNA, using the GenePak™ DNA PCR test kit. The PCR thermal profile was as follows: the first cycle of denaturation at 95°C for 60 s, annealing primers at 62°C for 40 s, and elongation at 74°C for 90 s. The second cycle consisted of denaturation at 95°C for 30 s, annealing primers at 60°C for 30 s, and elongation at 74°C for 60 s. This was followed by 43 cycles of denaturation at 95°C for 20 s, annealing primers at 58°C for 20 s, and elongation at 74°C for 40 s. After the last cycle, the tubes were incubated at 74°C for 60 s.

RESULTS

Among 65 Sistani breed animals, 11 positive and 5 doubtful results in the first PCR (fragment of the BLV gene was 347 bp) and 0 positive and 0 doubtful results in the second PCR (fragment of the BLV gene was 347 bp) were obtained. Among 120 animals belonging to the Yaroslavl breed, 21 positive and 7 doubtful results in the first PCR (fragment of the BLV gene was 347 bp) and 0 positive and 0 doubtful results in the second PCR (fragment of the BLV gene was 347 bp) were obtained. Among 50 animals belonging to the Mongolian breed, 4 positive and 1 doubtful results in the first PCR (fragment of the BLV gene was 347 bp) and 0 positive and 0 doubtful results in the second PCR (fragment of the BLV gene was 347 bp) were obtained. Among 35 animals belonging to the Black Pied breed, 6 positive and 2 doubtful results in the first PCR (fragment of the BLV gene was 347 bp) and 5 positive and 0 doubtful results in the second PCR (fragment of the BLV gene was 599 bp) were obtained (Figure 2).

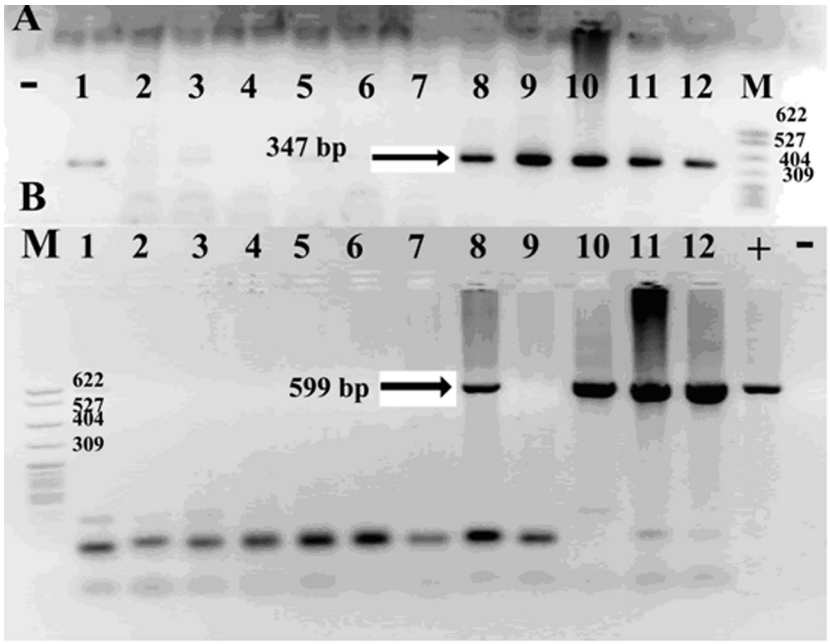

Figure 2. Electrophoresis of the bovine leukemia virus (BLV) gene fragment of 347 bp (PCR I, upper, **A**) and 599 bp (PCR II, lower, **B**) Black Pied breed. *Lane M* = DNA marker-PBR322/*Msp*I; + = positive control; - = negative control (PCR mixture without DNA). In the first PCR (upper): *lanes 4-7* = negative results; *lanes 1-3* = doubtful results; *lanes 8-12* = positive results. In the second PCR (lower): *lanes 1-7* and *9* = negative results; *lanes 8* and *10-12* = positive results.

DISCUSSION

The detection of BLV infection in cattle is carried out by virological methods-syncytial test (Ferrer et al., 1981; Otachel-Hawranek, 1993; Takahashi et al., 2004; Mingala et al., 2009), serological tests (Kozaczynska, 1999) and by molecular biology methods based on PCR (Naif et al., 1992; Klintevall et al., 1994; Marsolais et al., 1994; Czarnik et al., 2002; **González** et al., 2008). The implementation of a PCR method in BLV diagnosis could increase the detection number of infected animals. On the other hand, theoretical and experimental comparison of the available PCR primer sets for BLV detection has shown that the accuracy and reliability of PCR analysis depend on the primer quality (Limansky and Limanskaya, 2002). In the present study, among 270 animals, 42 positive and 15 doubtful results in the first PCR were found. Amplification of animals again by the second PCR was shown to be very effective in achieving reliability of BLV testing.

BLV infection is endemic in Argentina, especially in dairy herds of the central and northern areas. In highly infected herds, more than 60% of cows are seropositive to BLV (Ester et al., 1999; Amoril et al., 2009). In the United States, the estimated loss to the dairy industry due to BLV infection is reported to be more than $86 million annually (Da et al., 1993; Dus Santos et al., 2007). Among cows belonging to Polish Black-and-White breed, 60% positive results were obtained by PCR (Markiewicz et al., 2003).

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