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## Experimental transmission of Bovine leukemia virus in cattle via rectal palpation

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### Abstract

We examined whether Bovine leukemia virus (BLV) was transmitted by rectal palpation using a common sleeve between a BLV-infected cow and BLV-negative steers. Three of four steers developed antibodies against BLV as determined by agar-gel immunodiffusion (AGID) test between 7 to 10 weeks after the first rectal palpation using common sleeves from BLV-infected cow. In the steers, BLV proviral DNA were detected by PCR 1 to 5 weeks earlier than detection of the antibodies by the AGID test. Our experiments demonstrated that rectal palpation is a potential cause of BLV spread in herds and that detection of BLV proviral DNA in cattle by PCR is useful screening test for early diagnosis of BLV infection.

Key Words : Bovine leukemia virus, rectal palpation, transmission

Bovine leukemia virus (BLV) is a retrovirus that causes enzootic bovine leukosis, a persistent, lifetime infection in cattle. Most of the infected cattle do not show any clinical signs of the disease as an aleukemic (AL) state, about 30% of the infected cattle develop persistent lymphocytosis, and only 1-5% of the infected cattle develop malignant B cell lymphoma<sup>1)</sup>. Healthy AL cattle are asymptomatic

carriers and are recognized as a major source of transmission of the virus to susceptible cattle. Many field observations and experimental trials have indicated that whole blood containing lymphocytes infected with BLV is most important in the spread of BLV in cattle. Insect vectors may transfer BLV-infected blood cells mechanically to susceptible cattle and contribute to the maintenance of trans-

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mission within a herd<sup>2,5,12</sup>. Iatrogenic transmission could also occur through blood transfusion, and through the use of common injection needles as well as dehorning and castrating instruments<sup>3,16</sup>. Rectal palpation may be another route of iatrogenic transmission. Experimental infusion of large (500ml) or small (2ml) amounts of BLV-infected blood by rectal palpation resulted in seroconversion in the recipient cattle<sup>6,9</sup>. Rectal palpation of cattle is a common technique routinely performed in fertility control programs and is often performed in succession, using a common sleeve for several cows. Through this process the sleeve may be contaminated with bloody mucus, and infected blood may be transferred between individual cows. At a university dairy, a significantly greater rate of seroconversion was observed in cattle palpated with unwashed common sleeves than that observed in cattle palpated with sleeves washed between use<sup>8</sup>. In most commercial dairies, during artificial insemination, pregnancy diagnosis, and reproductive examination, cows are usually palpated more than four times a year. However, the risk of transmission of BLV using common palpation sleeves between cattle may not be a cause of concern. Therefore, the objective of this study was to determine whether BLV was transmitted to uninfected cattle which were palpated using a sleeve which had been used for palpating infected cattle.

The experiment was carried out at the Animal Research Center of Hokkaido from October to December 2003. One BLV-seropositive 16-year-old Japanese Black cow with a normal lymphocyte count (4,660/ $\mu$ l) was used as a healthy BLV-infected animal. In the BLV-infected cow, the percentage of BLV-infected cells in white blood cells was 30.8%. Six BLV-negative Holstein-Friesian steers that were one-year old were used in the transmission experiment. The steers were group

housed in a facility separated from the BLV-infected cow, and no insects were observed in the facility during the experimental period.

The BLV-infected cow was rectally palpated to the extent that some blood-contaminated mucus was attached to the polyethylene obstetrical sleeve. Four BLV-negative steers (H1, H2, H3, and H4) were palpated for more than 3 minutes immediately after the infected cow was palpated. The experimental rectal palpation was performed a total of 4 times, once weekly for 4 weeks. Turn of palpation in steers was at random. As a positive control, two BLV-negative steers (H5 and H6) were inoculated subcutaneously with 0.5ml of BLV-infected blood obtained from the BLV infected cow. Blood samples were obtained weekly for 10 weeks after the first rectal palpation or the inoculation with BLV-infected blood. The number of peripheral blood lymphocytes (PBL) was measured using an automatic blood cell counter (Cell Dyn 3700; Abbott).

BLV infection was determined by detection of serum antibodies against gp 51 of BLV by agar gel immunodiffusion test (AGID)<sup>11</sup> and of BLV proviral DNA by nested PCR. Genomic DNA was extracted from the blood using a Wizard genomic DNA purification kit (Promega) and used to amplify the BLV long terminal region (LTR) using primers BLV-LTR 1 (5' - TGTATGAAAGATCATGCCGAC - 3') and BLV-LTR533 (5' - AATTGTTTGCCGGTC TCTC - 3') in the first PCR and BLV-LTR256 (5' - GAGCTCTCTTGCTCCCGAGAC - 3') and BLV-LTR453 (5' - GAAACAAACGCGGG TGCAAGCCAG - 3') in the second PCR<sup>13,14</sup>.

To determine the percentage of BLV-positive cells in white blood cells of BLV-infected cattle, we amplified part of the BLV LTR region in the proviral genome and determined the amounts of products by real-time quantitative PCR system (Light Cycler Sys-

Table 1 . Detection of BLV infection in experimental cattle

| Experimental cattle <sup>a)</sup>                     | Diagnostic methods <sup>b)</sup> | Weeks after first procedure <sup>c)</sup> |   |   |   |   |   |   |   |   |   |    |   |
|---|----------------------------------|---|---|---|---|---|---|---|---|---|---|----|---|
|   |                                  | 0   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |   |
| Steers palpated with sleeves used on BLV-infected cow |                                  |   |   |   |   |   |   |   |   |   |   |    |   |
| H1  | AGID                             | -   | - | - | - | - | - | - | - | + | + | +  | + |
|   | PCR                              | -   | - | - | - | - | - | - | + | + | + | +  | + |
| H2  | AGID                             | -   | - | - | - | - | - | - | - | + | + | +  | + |
|   | PCR                              | -   | - | - | - | - | - | + | + | + | + | +  | + |
| H3  | AGID                             | -   | - | - | - | - | - | - | - | - | - | -  | + |
|   | PCR                              | -   | - | - | - | - | - | + | + | + | + | +  | + |
| H4  | AGID                             | -   | - | - | - | - | - | - | - | - | - | -  | - |
|   | PCR                              | -   | - | - | - | - | - | - | - | - | - | -  | - |
| Steers inoculated with BLV-infected blood             |                                  |   |   |   |   |   |   |   |   |   |   |    |   |
| H5  | AGID                             | -   | - | - | - | - | + | + | + | + | + | +  | + |
|   | PCR                              | -   | - | - | + | + | + | + | + | + | + | +  | + |
| H6  | AGID                             | -   | - | - | - | - | - | + | + | + | + | +  | + |
|   | PCR                              | -   | - | + | + | + | + | + | + | + | + | +  | + |

a) Four BLV-negative steers (H1-H4) were rectally palpated using sleeves from BLV-infected cow once weekly for 4 weeks. Two BLV-negative steers (H5, H6) were inoculated subcutaneously 0.5ml of BLV-infected blood.

b) Detection of antibodies against BLV by agar gel immunodiffusion test (AGID) and BLV proviral DNA by nested PCR.

c) Weeks after the first rectal palpation or the inoculation of BLV-infected blood.

tem ; Roche Diagnostic)<sup>14)</sup>. Genomic DNA was extracted from blood with a Wizard genomic DNA purification kit (Promega), and part of the BLV LTR was amplified in the presence of Light Cycler-FastStart DNA Master SYBR Green I (Roche Diagnostic), using primers BLV-LTR 256 and BLV-LTR 453. We also amplified the bovine gene for  $\beta$ -globin in each sample, using primers PC03 (5'-ACACAACGTGTTCACTAGC-3') and PC04 (5'-CAAC TTCATCCACGTTCCACC-3') to monitor the concentration of template DNA. Genomic DNA from FLK-BLV cells, which harbor six copies of the BLV genome, was serially diluted with total DNA from FLK cells, and samples were used as standard templates for calculations of percentage of BLV-positive cells.

In three of the four steers (H1, H2 and

H3) that were given rectal palpations with sleeves used on the BLV-infected cow, the antibodies against BLV were first detected between 7 and 10 weeks after the first rectal palpation. One steer (H4) did not develop antibody until 10 weeks after the first rectal palpation. In the positive control steers (H5 and H6), antibodies were first detected at 5 or 6 weeks after the inoculation of BLV-infected blood. After seroconversion, all steers remained positive for antibodies against BLV for the duration of the study (Table 1).

In the five steers shown to be BLV-positive by the AGID test, BLV proviral DNA were detected in the rectal palpation group at 5 or 6 weeks after the first rectal palpation and also detected in the control group at 2 or 3 weeks after the inoculation of BLV-infected

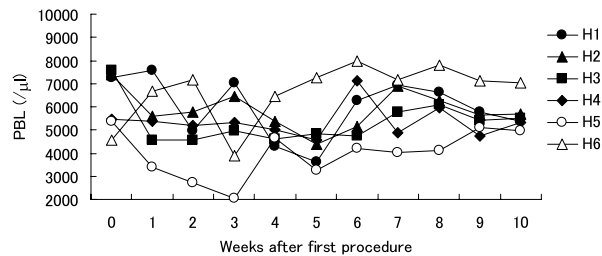


Fig. 1 . Changes of the numbers of peripheral blood lymphocytes (PBL) in experimental cattle. Four steers (H1-H4) were rectally palpated using sleeves from BLV-infected cow once weekly for 4 weeks. Two steers (H5, H6) were inoculated subcutaneously 0.5 ml of BLV-infected blood. Numbers at the bottom indicated weeks after the first rectal palpation or the inoculation of BLV-infected blood.

blood, and those remained BLV proviral DNA-positive for the duration of the study (Table 1). BLV proviral DNA was detected 1 to 5 weeks earlier than the first detection of antibodies. In steer H4 that did not develop antibody, BLV proviral DNA did not detected until 10 weeks after the first rectal palpation.

The numbers of PBLs in the experimental cattle are shown in Fig. 1. Two steers (H5, H6) in the control group showed a transient decrease of PBLs after the inoculation of BLV-infected blood, but a change of the numbers of PBL was not observed in four steers (H1-4) in the rectal palpation group.

We also examined the provirus load in the cattle by analyzing the percentages of BLV-positive cells (Fig. 2). The BLV-positive cells were first detected in three steers (H1, H2 and H3) at 6 weeks after the first rectal palpation and thereafter, the percentages of BLV-positive cells remained between 1.7 - 7.3% for 4 weeks. In two steers (H5 and H6) of control group, the BLV-positive cells detected at 5 or 2 weeks after the inoculation of BLV-infected blood and the percentages of BLV-positive cells of H6 increased to 19.7% to 10 weeks after the inoculation of BLV-infected

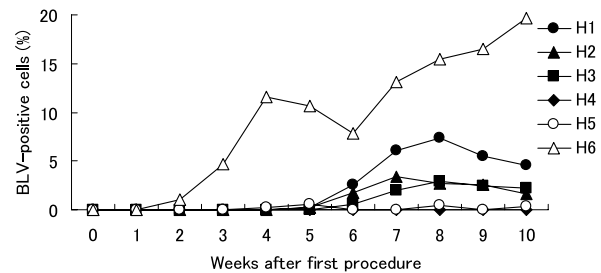


Fig. 2 . Changes of the percentage of BLV-positive cells in WBC of experimental cattle.

Four steers (H1-H4) were rectally palpated using sleeves from BLV-infected cow once weekly for 4 weeks. Two steers (H5, H6) were inoculated subcutaneously 0.5 ml of BLV-infected blood. Numbers at the bottom indicated weeks after the first rectal palpation or the inoculation of BLV-infected blood.

blood.

In the present study, three of four steers became infected with BLV after the experimental rectal palpation. Our results and previous studies<sup>8,9,15</sup> indicate that BLV can easily be transmitted between cattle by blood-contaminated sleeves. In our study, we were able to demonstrate transmission of BLV in cattle via rectal palpation on DNA level.

One of four steers was not infected with BLV, it might be due to a low dose of BLV-infected blood transferred by palpation sleeve. The dosage of BLV-infected blood (or infected lymphocytes) and the degree of trauma to the rectal mucosa may have been factors contributing to the transmission of BLV. Calves given the equivalent of 1 µl of BLV-infected blood via intramuscular, intravenous, subcutaneous, or intradermal routes developed infection<sup>4</sup>. Although rectal administration of 2 ml of BLV-infected blood to cattle without mucosal trauma could transmit BLV<sup>7</sup>, the minimum amount of blood needed to induce infection via the rectal route is unknown.

Several studies have observed that antibodies to BLV determined by the AGID test appeared in experimental cattle by 4 to 5

weeks after the rectal infusion of BLV infected blood<sup>6,15)</sup>. However, our experimental steers became seropositive between 7 and 10 weeks after the first rectal palpation. Because the virus titers transmitted to each steer by blood-contaminated sleeve were different, and it might be difficult to estimate that when steers infected with BLV.

BLV infection in cattle that were inoculated intramuscularly with BLV-infected blood could be detected as much as 2 to 4 weeks earlier by the use of PCR than by the use of the AGID test<sup>10)</sup>. In our study, BLV proviral DNA was detected by PCR in rectally palpated steers 1 to 5 weeks earlier than by the detection of antibodies using the AGID test. Therefore, detection of BLV proviral DNA by PCR would be a useful screening test to detect cattle that may be infected but not have the antibody.

We could not find the correlations of the number of PBL and the percentage of BLV-infected cells in BLV-infected cattle, however the difference in the level of BLV-positive cells of BLV-infected cattle may be associated with individual variability in the response to BLV infection.

Our experiments demonstrated that rectal palpation is a potential cause of BLV spread in herds and that detection of BLV proviral DNA in cattle by PCR is useful screening test for early diagnosis of BLV infection. In most commercial dairies, rectal palpations are performed frequently in routine fertility monitoring programs, and palpation sleeves are likely to become contaminated with blood in a manner visually undetectable. Therefore, in order to prevent the iatrogenic transmission of BLV, a clean disposable sleeve should be used for each cow during rectal palpation in herds. Early detection and eradication of BLV-infected cattle are also important for the prevention of BLV infection on a farm.

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