Bovine Leukemia Virus Infection in Japan:  
Antibody and Virus Detection in Cattle

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Abstract. The presence of antibody against bovine leukemia virus (BLV) was examined  
in dairy and beef cattle in Hokkaido and in the Towada district, Aomori Prefecture, by  
the immunodiffusion test in 1977 and in 1978, respectively. The percentage of antibody-  
positive cattle was higher in those cattle than in those observed in the previous survey  
(8.8% vs. 3.3% in Hokkaido and 44.2% vs. 32.2% in the Towada district), indicating the  
gradual increase of BLV infection in these parts of Japan. To clarify a relationship  
between the existence of BLV and the presence of antibody against BLV in cattle, syncyti-  
tum assay (SA) was performed with lymphocytes from cattle with or without BLV anti-  
body. All the cattle with antibody against BLV were positive for SA when bovine splenic  
cells were used as indicators, whereas 71.4% of them was positive when bovine thymic cells  
were used as indicators. These results suggest that BLV may be detected by SA from  
early all the cattle with antibody against BLV when appropriate cells are used as indi-  
cators. Fifteen percent of the antibody-negative cattle was positive for SA when bovine  
thymic cells were used and 17.6% positive when bovine splenic cells were used. Neither  
plaque nor inclusion body formation was observed in indicator cells which had been  
inoculated with lymphocytes. These SA-positive, antibody-negative cattle were considered  
to have been infected with BLV.

Since bovine leukemia virus (BLV) was detected from cattle with lymphosarcoma  
[8], several serological methods have been designed for detecting antibody against  
BLV [3, 4, 9, 10, 14]. The results of seroepizootiological surveys conducted in many  
countries indicate that there is wide-spread BLV infection in certain areas [1, 6, 7].  
The authors' serological survey revealed the dissemination of BLV infection in  
Hokkaido and in the Towada district of Aomori Prefecture. It is interesting to note  
that the frequency of appearance of antibody-positive cattle is as high in the Towada  
district as in any multiple-case herd of other countries examined [11].

This paper deals with (1) the results of  
serological surveys repeated at given intervals of time in the same areas of Hokkaido  
and the Towada district where the first survey was carried out, and (2) the relationship  
between the presence of BLV antibody and the existence of BLV in cattle. Syncyti-  
tum assay (SA) was employed as a new technique for the detection of BLV.

Materials and Methods

Sera: Serum samples were collected randomly  
from 2,829 cattle in 14 districts of Hokkaido in 1977.  
These cattle correspond 0.4% of all the cattle in  
each district. The samples had been obtained by  
the courtesy of Livestock Hygiene Service Centers,  
Prefecture of Hokkaido. Nearly all of them were  
from dairy (Holstein) cattle.

In the Towada district, serum samples were col-
lected from 647 beef cattle in eight pastures in 1978 and kindly provided by Dr. T. Yoshikawa of Kitasato University, Towada, Aomori.

Immunodiffusion test: The immunodiffusion test with glycoprotein antigen of BLV (gp-ID) was carried out as previously described [11, 14].

Syncytium assay: The procedure and the specificity of SA were described elsewhere [5]. Briefly, a monolayer of indicator cells which formed a 70 to 80% cell sheet was treated with DEAE-dextran for 1 hour and then incubated with 3 to 10×10^6 bovine lymphocytes to be tested. After 7 days of incubation, those indicator cells were fixed in methanol and stained with Giemsa stain. The appearance of an indicator cell having more than five nuclei was considered to be a positive syncytium formation.

Lymphocytes to be tested were isolated from fresh peripheral blood by Ficoll-Conray density gradient centrifugation [2]. At least 90% of the cells collected from the interface was identified as lymphocytes.

**Results**

The results of the survey conducted on cattle in Hokkaido and the Towada district were compared with those obtained from the previous survey (Tables 1 and 2).

In Hokkaido, 250 (8.8%) of 2,829 cattle were shown to have BLV antibody. The rate of reactors varied from 4.6 to 18.3% (averaging 8.8%) in 14 districts of Hokkaido. Relatively high positive rates were observed in the central part of Hokkaido: Shiribeshi, Hiyama and Hidaka districts (Table 1).

In the Towada district, 286 (44.2%) of 647 cattle had antibody and the rate of reactors varied from 4.4 to 66.5% (averaging 44.2%) in eight pastures (Table 2).

To examine the relationship between the presence of antibody against BLV and the virus, SA was used to detect BLV from lymphocytes of cattle with or without antibody against BLV. Most of the lymphocytes from cattle with this antibody were positive for SA (Table 3). The frequency of virus

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**Table 1. Comparison of results between serological survey on BLV antibody in cattle of Hokkaido conducted in 1974-1975 and that conducted in 1977**

<table>
<thead>
<tr>
<th>District</th>
<th>1977</th>
<th>1974–75&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of reactors/No. of tested</td>
<td>No. of reactors/No. of tested</td>
</tr>
<tr>
<td>Abashiri</td>
<td>25/409 6.1 19/433 4.4</td>
<td></td>
</tr>
<tr>
<td>Hidaka</td>
<td>22/120 18.3 4/150 2.7</td>
<td></td>
</tr>
<tr>
<td>Hiyama</td>
<td>19/116 16.5 2/97 2.1</td>
<td></td>
</tr>
<tr>
<td>Iburi</td>
<td>8/124 6.5 0/141 0</td>
<td></td>
</tr>
<tr>
<td>Ishikari</td>
<td>7/152 4.6 1/479 0.2</td>
<td></td>
</tr>
<tr>
<td>Kamikawa</td>
<td>11/120 9.2 4/134 3.0</td>
<td></td>
</tr>
<tr>
<td>Kushiro</td>
<td>22/359 6.1 21/299 7.0</td>
<td></td>
</tr>
<tr>
<td>Nemuro</td>
<td>47/360 13.1 16/250 6.4</td>
<td></td>
</tr>
<tr>
<td>Oshima</td>
<td>19/180 10.6 9/149 6.0</td>
<td></td>
</tr>
<tr>
<td>Rumi</td>
<td>10/152 6.6 4/150 2.7</td>
<td></td>
</tr>
<tr>
<td>Shiribeshi</td>
<td>11/100 11.0 0/68 0</td>
<td></td>
</tr>
<tr>
<td>Sohya</td>
<td>19/247 7.7 0/292 0</td>
<td></td>
</tr>
<tr>
<td>Sorachi</td>
<td>10/120 8.3 5/98 5.1</td>
<td></td>
</tr>
<tr>
<td>Tokachi</td>
<td>20/270 7.4 9/138 6.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250/2,829 8.8 94/2,878 3.3</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Data from the first survey on BLV antibody in cattle of Hokkaido [11].
Table 3. Results of SA conducted on cattle with or without BLV antibody

<table>
<thead>
<tr>
<th>BLV(+) antibody</th>
<th>Indicator cells used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymic cells</td>
</tr>
<tr>
<td>Positive</td>
<td>25/35(71.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>3/20 (15.0)</td>
</tr>
</tbody>
</table>

(a) Antibody status observed by gp-10 test.
(b) No. of cattle positive for SA/No. of cattle tested by SA.
(c) All cattle tested with bovine splenic cells were included in cattle tested with bovine thymic cells. Cattle positive for test with bovine splenic cells were also positive for test with bovine thymic cells.

detection, however, differed slightly with the indicator cells used in SA. When the antibody-positive cattle were examined, 71.4% and 100% of them were positive for SA when thymic and splenic cells, respectively, were used. On the other hand, 15.0% and 17.6% of the antibody-negative cattle were positive for SA when thymic and splenic cells, respectively, were used.

Discussion

As previously reported [11], the percentages of antibody-positive cattle detected by the first serological survey on dairy cattle in some areas of Hokkaido over a 1974–1975 period and on beef cattle in the Towada district in 1977 was 3.3% and 32.2%, respectively. The present survey which was conducted repeatedly in the same areas of Hokkaido in 1977 and in the Towada district in 1978 revealed a higher percentage of reactors (3.3% vs. 8.8% in Hokkaido and 32.2% vs. 44.2% in Towada). The increase of positive percentage suggests a gradual increase of BLV infection in cattle in Japan. Most of the beef cattle in the Towada district have been raised together in a pasture during summer. On the contrary, most of the cows in Hokkaido have been separately kept in their own herds throughout the year. The grazing style in a pasture may support the horizontal spread of BLV. The difference in positive percentage between the grazing styles used was also observed in a serological survey performed in the Hida area of Gifu Prefecture, Japan [12]. In this area, 52.1% of the cattle population put together in a pasture during summer was positive for antibody against BLV, but only 8.1% of the cattle kept separately in their own herds throughout the year was positive.

When carried out with bovine fetal thymic and splenic cells as indicator cells, SA was shown to be available for the detection of BLV in bovine lymphocytes. It is presumed that a majority of cattle which have antibody against BLV may carry BLV in their lymphocytes. In addition, if cattle to be tested have antibody, BLV will be detected by SA at a relatively high frequency.

Twenty cattle were positive for SA with splenic cells as indicators. Six of them, however, were negative for SA with thymic cells as indicators. In the authors' preliminary experiments, several transformed cell lines and normal embryonic cells were found to form syncytia when inoculated with cell-free BLV or BLV-infected lymphocytes, but these cells were different in sensitivity from one another (Onuma et al., unpublished data). Therefore, it is important in routine SA to select appropriate indicator cells.

Most of the lymphocytes from cattle without BLV antibody were negative for SA. Only a few of them were positive. Neither plaque nor inclusion body formation was observed when bovine thymic or splenic cells were inoculated with these lymphocytes. The existence of bovine syncytial virus infection has not yet been reported in Japan. Onuma and Olson (1977) reported that BLV antigen could be detected in cultured lymph nodes from
cattle having no detectable antibody against BLV. Therefore, these positive cases may be attributed to infection with BLV.

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References


要 約

わが国におけるウシ白血病ウイルス感染（牛からの抗体およびウイルスの検出：本間利夫・小沼 操・見上 懸・伊沢久夫（北海道大学獣医学部家畜伝染病学講座））について、北海道および青森県十和田地方のウシを対象に、ウシ白血病ウイルス（BLV）に対する抗体の保有状況を調査した。その結果、北海道（1977年調査）では8.8%が、また十和田地方（1978年調査）では44.2%がBLV抗体陽性であった。今回得られた抗体陽性率は前回の抗体調査で得られたそれと比べて高く、わが国においてもBLVによる汚染は徐々に進行していることがうかがえた。また、ウシにおけるBLV抗体保有とBLV保有との関係を明らかにするために、syncytium法を応用して、BLV抗体陽性牛と陰性牛のリンパ球からBLVの検出を試みた。その結果、BLV抗体陽性牛ではindicator細胞としてウシ胎児胸腺細胞を用いるときには71.4%が、またウシ胎児胸腺細胞を用いるときには全例が、syncytium法陽性と判定された。この成績は、BLVに対する抗体を保有するウシのほぼ全例が、リンパ球にBLVを保有することを示唆するものと思われる。