

# A serological survey of bovine leukemia virus infection in dairy cattle in the suburban farming area of Japan

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## 日本都市近郊酪農場 乳牛의 牛白血病感染에 대한 血清學的 調查研究

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抄錄: 1983년부터 1985年度까지 사이에 日本 Saitama縣 各小都市近郊의 酪農場에서 飼育되는 2,407頭의 乳牛를 對象으로 BLV(bovine leukemia virus) 感染與否를 血清學的檢査(agar-gel immunodiffusion test)를 實施한 結果 平均陽性率은 4.9%이었고, 年齡이 增加함에 따라 2.6%에서 부터 9.8%까지 陽性率이 있으며, 同一地域에서도 調査酪農場別로 陽性率은 큰 差가 있었다.

本調査研究의 結果를 BLV感染에 대한 獸醫疫學的 基礎資料로 提示하는 바이다.

**Key words:** bovine leukemia virus, dairy cattle, serological survey.

### Introduction

Enzootic bovine leukemia (EBL) is caused by bovine leukemia virus (BLV). It is a neoplastic disease of the lymphoid tissue, in which lymphocytes are primarily affected.<sup>1,2</sup> In recent years, the occurrence of EBL was evidenced by increased BLV antibody positive rates in some districts in Japan.<sup>3-10</sup> Surveys have been made to determine the distribution of BLV antibodies in cattle as a first step toward eradicating the disease.<sup>11</sup> The present survey on BLV antibody was conducted in typical suburban dairy cattle raised in the eastern part of Saitama prefecture.

### Materials and Methods

**Sera:** Serum samples were collected from 2,407

Holstein cows raised in the eastern part of Saitama prefecture over a period from 1983 to 1985 (Fig 1).

**Antigen:** The antigen for the ID test was prepared from culture fluids from a BLV-infected cell line (FLK; fetal lamb kidney). Briefly, culture fluids were centrifuged at 66,000 for 90min, and the supernatant was concentrated 100 times by dialysis against polyethylene glycol. This concentrated preparation contained BLV antigen composed of ether-resistant internal protein antigen (p antigen) and ether-sensitive glycoprotein antigen (gp antigen).

**Agar gel immunodiffusion (ID) test:** Tests were run with 1% agarose prepared with 0.05M tris-HCl buffer (pH 7.2) containing 8.5% NaCl. Positive serum obtained from a cow with lymphosarcoma was placed in two peripheral wells as a control. Wells were filled only once and incubated at room

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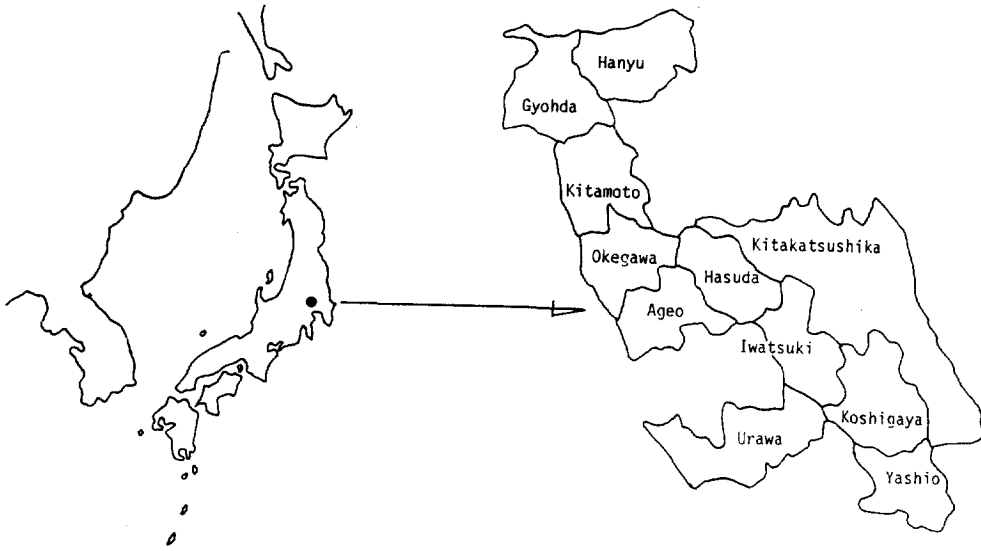


Fig 1. Sampling sites of bovine sera in Saitama prefecture

temperature in a humidified chamber. At 72 hours of incubation, the results were read.<sup>6</sup>

**Statistical Analysis:** The chi-square test was employed for a comparison of the positive rate between test groups of cattle.

### Results

Of 2,407 serum samples, 119(4.9%) were positive for BLV antibodies. Of 119 positive samples, 117 were reactive to gp antigen and 2 to both gp and p antigens. There was no serum sample which reacted only to p antigen.

The rate of positive samples varied from 2.6 to 9.8% among different age groups of cattle from younger than one year to 14 years. It increased with age (Table 1).

Serum samples were collected from cattle raised in 10 cities and 1 country. The positive rate was 0% in Iwatsuki and Koshigaya, 2.2~2.9% in Hanyu, Kitakatsushika, Okegawa, and Yashio, 4.2~4.4% in Gyoda and Urawa, 7.5~7.6% in Ageo and Hasuda, and 21.1% in Kitamoto. Thus, large differences were observed among the districts (Table 2).

In Kitamoto, where the highest rate was obtained, positive cattle were found in 2 of 5 farms surveyed. Particularly in one of the 2, the positive rate was

as high as 85.7%. In Ageo, positive cattle were found in 8 of 30 farm. No positive cattle were observed in the remaining 22 farms. There was no specific tendency in geographical distribution of positive cattle in either Kitamoto or Ageo area (Table 3 and 4, Fig 2 and 3).

Table 1. Age distribution of BLV-positive cattle raised in the eastern region of Saitama prefecture

Age (in years)	No of cattle tested	No of positive cattle	Positive rate (%)
<1	71	2	2.8
1	137	5	3.6
2	304	8	2.6
3	429	11	2.6
4	398	18	4.5
5	355	21	5.9
6	270	22	8.1
7	182	13	7.1
8	131	11	8.4
9	61	6	9.8
10	33	1	3.0
11≤	36	1	2.8
Total	2,407	119	4.9

**Table 2.** Geographical distribution of BLV-positive cattle raised in the eastern region of Saitama prefecture

District	No of cattle tested	No of positive cattle	Positive rate (%)
Ageo	566	43	7.6
Gyoha	182	8	4.4
Hasuda	200	15	7.5
Hanyu	142	4	2.8
Iwatsuki	46	0	0
Kitakatsushika	646	19	2.9
Koshigaya	42	0	0
Kitamoto	76	16	21.1
Okegawa	312	7	2.2
Urawa	120	5	4.2
Yashio	75	2	2.7
Total	2,407	119	4.9

**Table 3.** Farm distribution of the positive reactors in Kitamoto district of Saitama prefecture

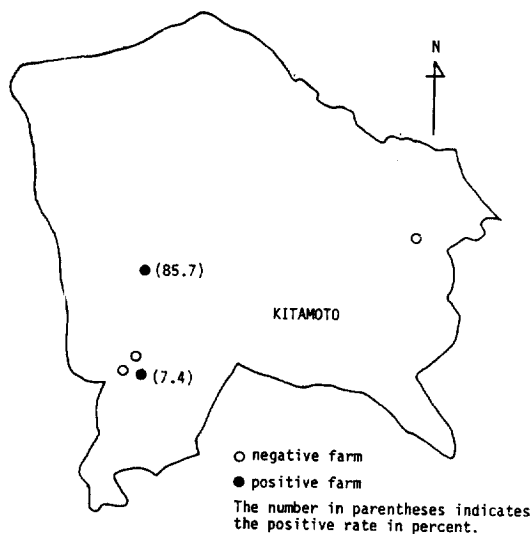
Farm	No of cattle tested	No of positive cattle	Positive rate (%)
KU	14	12	85.7
KA	8	0	0
EN	7	0	0
KI	20	0	0
UC	27	2	7.4
Total	76	16	21.1

**Table 4.** Farm distribution of the positive reactors in Ageo district of Saitama prefecture

Farm	No of cattle tested	No of positive cattle	Positive rate (%)
EN	76	9	11.8
HA	8	2	25.0
IC	21	5	23.8
HI	37	1	2.7
AR	46	2	4.3
SU	30	9	30.0
SI	16	10	62.5
OG	9	5	55.6
Other 22 farms	323	0	0
Total	566	43	7.6

## Discussion

Two modes of infection are proposed for BLV infection. One is vertical transmission, such as maternal or lactic transmission, in which BLV is transmitted from dams to fetuses or newborn calv-

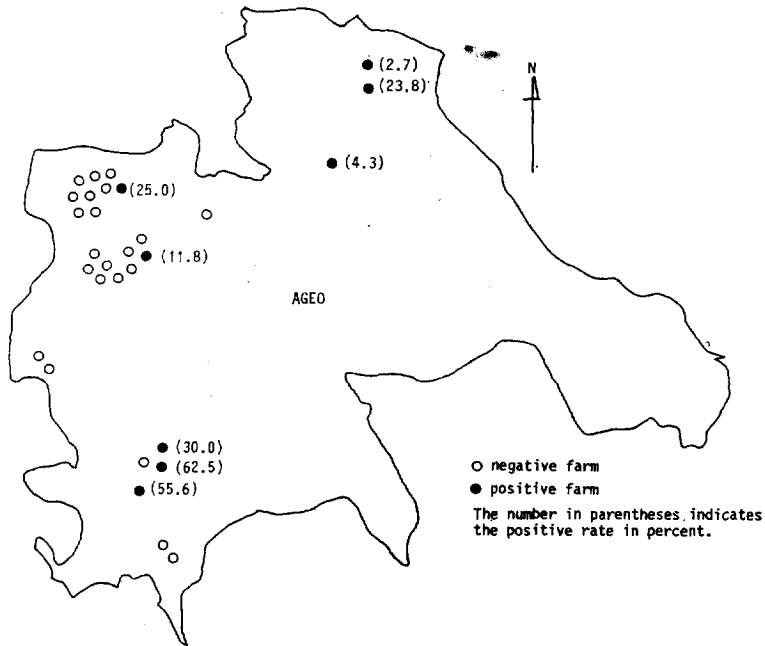


**Fig 2.** Sampling sites of bovine sera in the survey of BLV antibodies in Kitamoto district, Saitama prefecture

es.<sup>12-17</sup> The other is horizontal transmission, such as mechanical transmission of BLV by hematophagous insects like *Tabanus trigeninus* or *Tabanus nipponicus* and artificial transmission caused by injection with a contaminated needle, and so on.<sup>18-23</sup> In the present survey, no useful information was obtained in regards to the mode of transmission. However, the results indicate that many cattle were subclinically infected with BLV.

Previous studies suggested that the positive rate is lower in cattle kept in barns throughout the year than in cattle released in pasture during summer.<sup>7,24</sup> Cattle kept in barns were considered to be less exposed to horizontal transmission of BLV by hemophagous insects than cattle released in pasture. The cattle subjected to the present study were kept in barns for all seasons. Nevertheless, the rate of positive samples was comparable to the national average rate of 3.7%<sup>25</sup> and 4.2%<sup>26</sup> in 1980 and 1982, respectively, and to the average rate of 3 cities in Saitama prefecture or 5.2%.<sup>27</sup> The results suggest that intra-barn transmission may occur more frequently than expected when BLV is present in the building.

The close relationship between the positive rate for EBL and breeding environmental factors has



**Fig 3.** Sampling sites of bovine sera in the survey of BLV antibodies in Ageo district, Saitama prefecture

been pointed out in the national serological survey. Even in the present small-scaled survey in Saitama prefecture, the difference in the positive rate was observed among districts studied. The positive rate in Kitamoto was as high as 21.1%, while no positive cattle were observed in Iwatsuki or Koshigaya. In districts where positive cattle were observed, they were concentrated in some limited farms. For example, in Kitamoto, positive cattle were observed in 2 of the 5 farms, and one of the farms showed a markedly high rate of 85.7%. In Ageo, positive cattle were found only in 8 of the 30 farms, and the positive rate widely varied from 2.7 to 62.5% among the positive farms. Thus, even in this local survey, an enzootic nature of BLV infection was obviously recognized.

From the standpoint of classification by the age group, the positive rate was low (2.6~3.6%) in cattle up to 3 years of age, while it was 4.5% in the group of cattle 4 years of age. The positive rate increased gradually with age, reaching 9.8% in the group of cattle 9 years of age. The BLV-antibody positive rate has been reported to increase with older age.<sup>9,28</sup> The correlation between the

positive rate and age was confirmed even in this survey. Once infected with BLV, the host carry the virus or viral genome in the host lymphocytes throughout life, but the antibody titer temporarily decreases at the time of parturition and some other occasions.<sup>28,29</sup> Therefore, the positive rate increases in cattle with age. Owners reluctant to cull positive cows also help increasing positive reactors among older cattle.

Vaccination seems to be an ideal preventive measure against BLV infection at the time of epidemic spread. The investigation of vaccine, however, is still at the stage of exploration.<sup>13,30,31</sup> Elimination and isolation of infected cattle may be practical to prevent the spread of BLV infection. In some European countries, the method have already been applied, and BLV-positive cattle have been eliminated legally. In a follow-up survey the positive rate was 30.0% on the YA farm in 1983, but decreased to 4.0% on the same farm in 1986 as the results of their eradication effort (Table 5).

It was estimated that the decrease in the positive rate after 3 years was due to the successive planned culling of positive cattle and the introduction of

**Table 5.** Positive reactors in two farms in 1983 and 1986

Farm	Date of serum collected	No of cattle tested	No of positive cattle	Positive rate (%)
K U	July 1983	14	12	85.7
	Apr 1986	25	19	76.0
Y A	July 1983	30	9	30.0
	Apr 1986	25	1	4.0

negative cattle into this farm. On the other hand, on the KU farm where no measures had been taken for BLV clearance, the rate was 85.7 and 76.0% in 1983 and 1986, respectively. Moreover, this farm has experienced 2 occurrences of EBL before. These facts prove that a herd of cattle, including cattle with EBL, shows a high positive rate.<sup>24</sup> Some reports indicated the eradication of BLV only by culling of positive cattle.<sup>9,32</sup> It is difficult to raise BLV-positive cattle separately from the other cattle in suburban dairy farming area. It is also difficult for financial reasons to cull all positive cattle at one time. Under these circumstances, planned successive culling of positive cattle and introduction of negative cattle seem to be practical procedures for BLV clearance until a more effective measure for the prevention found.

### Summary

A survey on the prevalence and distribution of antibodies to BLV was performed by the agar-gel immunodiffusion test over a period from 1983 to 1985. More than 2,407 serum samples were collected from Holstein cattle raised in the eastern part of Saitama prefecture where suburban dairy farm is operated. The average positive rate of this period was 4.9%. The rates of reactive samples varied from 2.6 to 9.8% among the age groups of cattle from younger than one year to 14 years of age. The positive rate increased gradually with age. The positive rates also varied widely from 0 to 21% among areas surveyed. Furthermore, there were large differences in this rate among farms even in the same area. The results were interpreted and discussed in connection with the enzootic feature of BLV infection.

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