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## **Distribution of Antibodies to Bovine Leukemia in Japanese Black Cattle Raised in the Southern Region of Japan**

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### **Summary**

Serum samples were collected from 624 Japanese black cattle in the southern region of Japan during a period time of from 1982 to 1984, and antigen titers were determined with bovine leukemia virus (BLV) antigen by the agar-gel immunodiffusion test. An over-all average positive rate against BLV was 23.9% during this period, however, a tendency to increase year by year. Generally, older individuals showed higher positive rates than younger ones. Antibody-positive rates varied according to the size of farm: a larger herd tended to have higher positive rates. Of 42 paired sera of dam and calf living together, the presence of BLV antibodies were detected in both dam and calf in 5 pairs (11.9%), in dam only in 6 pairs (14.5%), and in calf only in 3 pairs (7.1%), respectively. These results indicated an extensive spread of BLV among Japanese black cattle in the region.

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## Introduction

Bovine leukemia virus (BLV) causes enzootic bovine leukemia (EBL), a neoplastic disease of lymphoid reticular tissues. Incidence of the disease generally seems increasing, and there are some regions where the antibody-positive rates are unusually high (Fujikawa *et al.*, 1979; Ishihara *et al.*, 1979; Honma *et al.*, 1980; Okada *et al.*, 1981; Ohshima *et al.*, 1983). Environmental contamination with BLV appears to be spreading in Japan, and there is an urgent need to control EBL. This paper deals with a survey on the prevalence and distribution of BLV antibody in Japanese black cattle raised in the eastern part of Kagoshima Prefecture, where Japanese black cattle are primarily bred.

## Materials and Methods

Sera. Serum samples were collected from 624 Japanese black cattle in the four towns, Ariake, Ohsaki, Ohsumi and Kushira, over a period from July to September every year in 1982, 1983, and

1984. These towns are located in Soh County, Kagoshima Prefecture, in the southern region of Japan (Fig. 1). The collected serum samples were stored at  $-20^{\circ}\text{C}$  before testing.

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

Agar gel Immunodiffusion (ID) Test. The test was run with 1% agarose prepared with 0.05M tris-HCl buffer (pH 7.2) containing 8.5% NaCl. Positive serum obtained from cattle with lymphosarcoma was placed in two peripheral wells as a control. Wells were filled only once and

incubated at room temperature in a humidified chamber. At 72 hours of incubation, the results were read. (Ohshima et al., 1980).

Statistical Analysis. The chi-square test was employed for a comparison of the positive rate of antibody against BLV between any two groups of cattle.

## Results

The Positive Rate of Antibody Against BLV in each Year.

The average positive rate was 23.9% for 3 years, but the rate increased year by year: 12.6% in 1982, 21.7% in 1983, and 34.9% in 1984 (Table 1).

The Positive Rate and Age Groups.

The positive rate was 17.6% in cattle younger than one year, and 17.4% in those of one year-old age. It increased with the increase in age, reaching 28.6% in cattle of 4 years-old. The positive rate decreased in cattle 5 years of age and older (Table 2).

The Positive Rate and GeoGraphy.

The positive rate was 27.3% in Ohsaki, 24.0% in Ariake, 10.3% in Ohsumi, and 19.2% in Kushira. Thus, the rate varied from town to town (Table 3).

The Positive Rate and the Scale of Breeding.

The Rate of Reactive Sera Collected from Pairs of Dams and Calves Lodged Together.

Of 42 paired sera collected from dams and their calves, 28 (66.7%) were negative for BLV antibody as a pair. Both dams

Twenty farms were randomly selected from four towns and distributed to 3 groups by the scale of breeding. The antibody-positive rate was as low as 19.4% in a group of farms raising less than 5 cattle each, 27.5% in a group raising 6 - 10 cattle each, and as high as 25.8% in a group raising 11 cattle or more each. Thus, the farms operated on a larger scale showed a higher positive rate (Table 4).



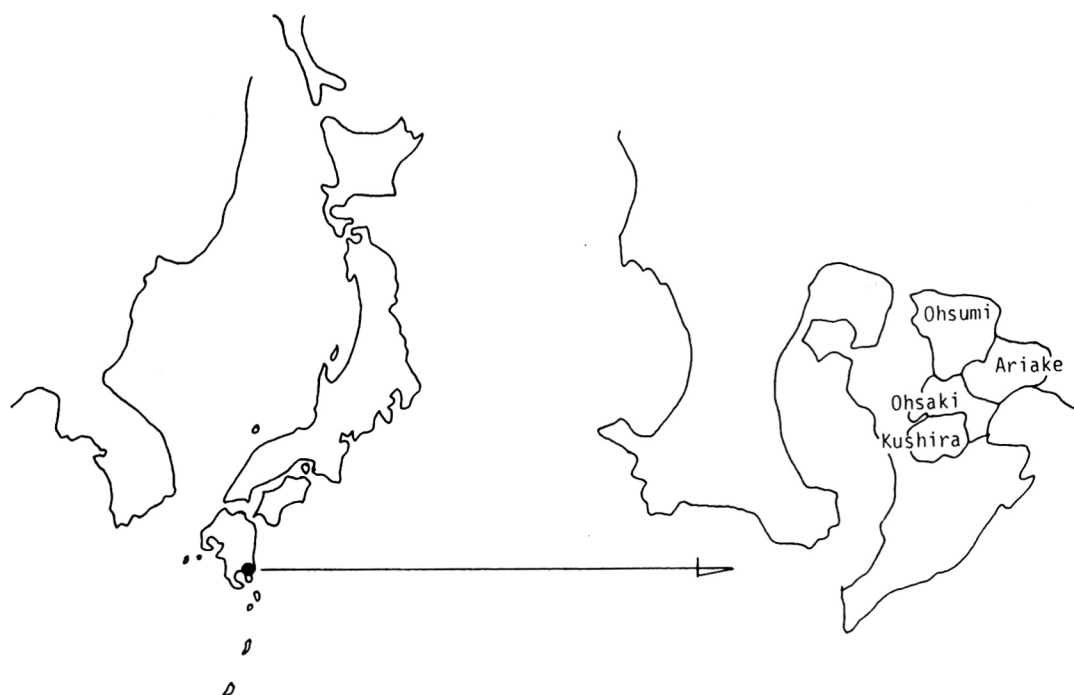


Fig. 1 Sampling sites of bovine sera in Kagoshima Prefecture.

Table 1 Annual distribution of BLV antibodies in Japanese black cattle in the southern region of Japan.

Serum collection	No. of cattle tested	No. of positive cattle	Positive rate (%)
1982	191	33	17.4
1983	267	58	21.7
1984	166	58	34.9*
Total	624	149	23.9

\* Significantly different from 1982 or 1983 by the chi-square test at  $p < 0.001$ .

**Table 2** Age distribution of the positive reactors in Japanese black cattle raised in the southern region of Japan.

Age (years)	No. of cattle tested	No. of positive cattle	Positive rate (%)
< 1	51	9	17.6
1	23	4	17.4
2	76	19	25.0
3	59	15	25.4
4	70	20	28.6
5-6	125	32	25.6
7-8	86	21	24.4
≥ 9	134	28	20.9
Total	624	149	23.9

**Table 3** Geographical distribution of the positive reactors in Japanese black cattle raised in the southern region of Japan.

District	No. of cattle tested	No. of positive cattle	Positive rate (%)
Ariake	383	92	24.0
Ohsaki	176	48	27.3*
Ohsumi	39	4	10.3
Kushira	26	5	19.2
Total	624	149	23.9

\* Significantly different from Ohsumi by the chi-square test at  $p < 0.001$ .

Table 4 Distribution of the positive reactors based  
on the herd size / farm.

No. of cattle on farm	No. of farms examined	No. of cattle tested	No. of positive cattle	Positive rate (%)
< 5	8	31	6	19.4
6 - 10	11	80	22	27.5
> 11	4	62	16	25.8
Total	23	173	44	25.4

Table 5 The results of the AGI test with paired sera of dams  
and their calves in the southern region of Japan.

<u>Results of AGI test in</u>		No. of pairs of dam and calf (%)
Dams	Calves	
-	-	28 ( 66.7)
+	-	6 ( 14.3)
+	+	5 ( 11.9)
-	+	3 ( 7.1)
Total		42 (100.0)

and their calves were positive in 5 pairs. In 6 pairs only dams were positive, while in 3 pairs only calves were positive.

### Discussion

The average positive rate of antibody against BLV in the 3 years was 23.9% in the four towns. The value is lower than rates reported before in Japanese black cattle, such as 46.7% in Iwate Prefecture, (Ohshima *et al.*, 1984) 60.7 and 59.2% in Gifu Prefecture, (Ishihara *et al.*, 1979; Ohshima *et al.*, 1982). On the other hand, the rate is markedly higher than the rates reported in Shimane Prefecture (3.2%) (Kawahira *et al.*, 1982) or in Hokkaido Prefecture (3.3%). (Ohshima *et al.*, 1980) Therefore, the present survey indicates that contamination with BLV is prevalent even in the four towns.

It has been reported that the positive rate is higher among cattle grazing in pasture than those kept in barns, and that reactors increase in numbers each grazing period. Hence, the rate seems to differ de-

pending on the breeding practice. (Ishihara *et al.*, 1979; Ohshima *et al.*, 1981; Kawahira *et al.*, 1982; Ohshima *et al.*, 1983) The possible reason may be that cattle grazing in pasture have more chances of close contact with one another than those kept in barns. In this case, horizontal transmission of BLV seems to play an important role in spreading the virus. (Onuma *et al.*, 1979). The primary vector of BLV is a gadfly which transmits infected lymphocytes mechanically to achieve infection. (Onuma *et al.*, 1977, Onuma *et al.*, 1978; Bech-Nielsen *et al.*, 1978; Onuma *et al.*, 1980). Of towns under the survey, Ariake and Ohsaki town are located in a mountainous area where gadflies are plentiful. Furthermore, most of the cattle in these towns are kept on a pasture during daytime. Because of these facts, the higher rate of reactors may have been obtained from these towns than from towns like Ohsumi and Kushira located on flat plains. In these towns, cattle are mostly kept in barns.

An extreme enzootic feature of BLV contamination was confirmed in the present study. Pastures raising a large number of cattle showed a higher positive rate than those raising smaller numbers. This finding agrees with the previous report indicating that the rate of BLV reactors increased in proportion to the scale of breeding. (Fujikawa *et al.*, 1979; Ichijo *et al.*, 1982, Kawahira *et al.*, 1982). Infection is likely to occur in a large-scaled pasture, because there are many susceptible cattle, or because there are more chances to introduce both and infected susceptible cattle. Besides horizontal transmission mentioned above, (Piper *et al.*, 1975) vertical transmission is also supposed to take part in BLV infection. (Rosenberger, 1963, Ferrer *et al.*, 1976, Ohshima *et al.*, 1978). This supposition was supported particularly by the fact that BLV was isolated from some calves born from dams infected with BLV before the intake of colostrum. (Onuma *et*

*al.*, 1979; Kono *et al.*, 1987; Thurmond *et al.*, 1983). In addition, papers were published to report an experimentally induced intrauterine infection and an existence of maternal antibody. (Onuma *et al.*, 1975; Burrige *et al.*, 1982). Whatever routes were used for transmission, conversion occurred in cattle about one month after the inoculation of BLV. (Miller *et al.*, 1981). In the present survey, three calves were found positive for BLV antibody, even though their corresponding negative dams were housed together. Since these calves were all older than 2 months at that time, these antibodies to BLV were attributed to active infection. In order to find out the precise time of infection, a continuous close observation must begin right after the parturition.

The rate of BLV- infection in herd increases with the advance in age. (Ohshima *et al.*, 1980; Kawahira *et al.*, 1982; Ohshigawa *et al.*, 1982). The

results obtained from this survey were in agreement with this point. Generally, cows of Japanese black breed, require a long period for breeding. This may be one of the factors responsible for persistence of BLV in the region, because breeding increases chance to have contact with infected individuals. From the standpoint of preventive veterinary medicine, there are some reports on the successful clearance of area from BLV infection cattle by isolation and culling of exposed cattle. (Yoshigawa *et al.*, 1982; Ohshima *et al.*, 1983). Such eradication effect is highly desirable in the region surveyed.

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# บทย่อ

## ภูมิคุ้มกันต่อโรค โปวาย ลิวคีเมีย ในวัวดำ ที่เลี้ยงในภาคใต้ของประเทศไทย

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เวชศาสตร์สัตวแพทย์ 18(3); 2531:229-240

ได้เก็บตัวอย่างซีรัมจากวัวดำของญี่ปุ่นจำนวน 624 ตัว ในภาคใต้ของประเทศไทย ระหว่างปี พ.ศ. 2525 ถึง 2527 แล้วนำมาหาปริมาณของแอนติบอดีที่มีต่อแอนติเจนของไวรัสโปวายลิวคีเมียโดยวิธีเจลมิวโนดิฟฟูชัน พบว่าซีรัมให้ผลบวก 23.9% ในช่วงระยะเวลานี้ อย่างไรก็ตามมีแนวโน้มว่าจะเพิ่มขึ้นทุก ๆ ปี โดยทั่วไปวัวที่มีอายุน้อยกว่ามีแอนติบอดีสูงกว่าวัวที่มีอายุน้อยกว่า อัตราของแอนติบอดีที่ให้ผลบวกแตกต่างกันไปตามขนาดของฟาร์ม ฟาร์มใหญ่มักให้ผลบวกในอัตราที่สูงกว่า พบว่าซีรัมของแม่วัวและลูกวัวที่อยู่รวมกันจำนวน 42 คู่ มีแอนติบอดีต่อไวรัสโปวายลิวคีเมียในทั้งแม่วัวและลูกวัวจำนวน 5 คู่ (11.9%) ในแม่วัวอย่างเดียวจำนวน 6 คู่ (14.5%) และในลูกวัวอย่างเดียวจำนวน 3 คู่ (7.1%) จากผลเหล่านี้ชี้ให้เห็นว่ามีการแพร่ของไวรัสโปวายลิวคีเมียในวัวดำของญี่ปุ่นอย่างกว้างขวางในภูมิภาคแถบนี้

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