Seroepizootiological Survey on Antibodies against Bovine Leukemia Virus in Japanese Black Cattle

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Abstract. A seroepizootiological survey was performed on antibodies against bovine leukemia virus (BLV) in beef cattle in the Hida district by using the immunodiffusion test with BLV glycoprotein antigens. These cattle were maintained in two different herd styles. In one style, cattle from various herds were placed together on the same pasture during summer. In the other, cattle were kept in their own herds throughout the year. More than one-half (52.1%) of the former cattle was positive for BLV antibodies, but only 8.1% of the latter cattle was positive. Reactors increased gradually in number with the advance in age. Eighty-eight percent of the animals which had shown lymphocytosis in the hematological test was positive for BLV antibodies, whereas 38.3% of the animals free from lymphocytosis was also positive. These results indicated that there was a relationship between BLV infection and the incidence of lymphocytosis.

Enzootic bovine leukosis is a contagious disease [1, 14] induced by bovine leukemia virus (BLV). Recent seroepizootiological surveys have shown that BLV infection is widespread in European countries and the United States [5-7, 12]. In Japan, rather widespread infection with BLV was also suggested in a preliminary local survey on Japanese Shorthorn (beef) cattle in the Towada district, Aomori Prefecture [9]. This survey indicated a need for further investigation of beef cattle in Japan.

In the Hida district, Gifu Prefecture, 45 cases of bovine leukosis were found during a 10-year period from 1969 to 1978. Of them, 17 cases were detected in T village [2]. The authors have previously reported hematological diagnostic criteria [4] and comparison between hematological status and

serological condition in Japanese Black cattle in T village [3]. A preliminary survey in T village revealed that BLV infection was widespread.

The present paper describes a seroepizootiological survey on 615 Japanese Black cattle in the Hida area in relation to the different herd styles.

Materials and Methods

Sera: Serum samples were collected randomly from 585 Japanese Black (beef) cattle in the Hida district, Gifu Prefecture, in 1977 and 1978, as shown in Fig. 1. Serum samples were collected from 265 cattle in 4 areas (a to d) of T village. These cattle occupied 38.2% of all the beef cattle in the 4 areas. Serum samples were also collected randomly from about 1.0 to 5.4% of all the cattle kept in some other areas. Thirty serum samples were also collected from beef cattle in H town, which is located outside the Hida district.

Fig. 1. Outline map of the Hida district in Gifu Prefecture showing the location of the 9 areas sampled

Hematological examination: Total leukocyte counts were done with a Coulter counter. Smears for differential counting were made from all the samples. Results were evaluated according to the key for Japanese Black cattle [4].

Immunodiffusion test: The immunodiffusion test with glycoprotein antigens (gp-ID) was performed, as previously described [8-10].

Herd style: Conventional style—In the T, A and Ku areas, most farmers had 3 to 7 Japanese Black cattle. Most of the cattle in these areas were breeding cattle which had been raised together in the same pasture during summer (May to October) and kept in each farmer's barn during winter.

Modern style—In the other areas, most farmers had more than 10 beef cattle which were kept most-

Table 1. Reactors to hematological and serological tests in cattle from T village

Area	Positive for	
	Hematological test (%)	Serological test (%)
a	9/91 ^{a)} (9.9)	53/92 ^{a)} (57.6)
b	15/42 (35.7)	25/42 (59.5)
c	8/45 (17.8)	18/45 (40.0)
d	8/68 (11.8)	61/86 (70.9)
Total	40/246 (16.3)	157/265 (59.2)

Remarks.

^{a)} No. reacted/No. tested.

ly in feedlot herds throughout the year.

Therefore, cattle from herds of conventional style had more chance to contact other cattle than cattle from herds of modern style.

Results

Since 17 cases (38%) of leukosis in the Hida district were found in T village, blood samples were collected from 265 cattle in the 4 areas of T village and examined hematologically and serologically (Table 1). From the hematological examination, 40 (16.3%) cattle showed lymphocytosis, and the rate of positive animals in each area varied from 9.9 to 35.7%. About 60.0% of the cattle was positive for antibodies against BLV. The rate of reactors in each pasture varied from 40.0 to 70.9%.

The results of the seroepizootiological

Table 2. Different reactors to serological test in cattle from different herd styles in the Hida district

Location	Modern style ^{a)}	Location	Conventional style ^{a)}
	Reactors (%)		Reactors (%)
K village	2/30 ^{b)} (6.7)	T village	157/265 (59.2)
N village	6/50 (12.0)	A village	6/30 (20.2)
S station	3/30 (10.0)	Ku village	12/41 (29.3)
H town ^{c)}	0/30 (0)		
Ka village	5/40 (12.5)		
Ta city	1/30 (3.3)		
Total	17/210 (8.1)		175/336 (52.1)

Remarks.

^{a)} See Materials and Methods of the text.

^{b)} No. reacted/No. tested.

^{c)} Outside of the Hida district.

Table 3. Different reactors to serological test in cattle from different age groups

Age (years)	Reactors	%
0-1	7/54 ^{a)}	13.0
2-4	35/148	23.6
4-6	39/93	41.9
6-8	29/57	50.9
>8	61/131	46.6
Total	171/483	35.4

Remarks.

^{a)} No. reacted/No. tested.**Table 4.** Hematological status of cattle and correlation with serological results

Hematological ^{a)} status	Number of cows	(%)	Serological result (%)
Normal	504	(82.0)	193/504 ^{b)} (38.3)
Suspect	86	(14.0)	61/86 (70.9)
Lymphocytosis	25	(4.1)	22/25 (88.0)
Total	615		276/615 (44.9)

Remarks.

^{a)} According to the Key for Japanese Black Cattle [2].^{b)} No. reacted/No. of cows classified by the Key for Japanese Black Cattle.

survey on BLV antibodies in the Hida district were grouped according to the herd style, as shown in Table 2. Of 336 cattle in T, A and Ku villages where cattle were put together on the same pasture during summer (conventional style), 175 (52.1%) cattle were positive for BLV antibodies. A few reactors (8.1%), however, were found in cattle from the other areas where cattle were kept in their own herds throughout the year (modern style).

When the results of serological examination were analyzed by age group, a relatively few reactors were found in cattle less than 2 years old, and the number of reactors increased gradually with the advance in age. Nearly one-half of the cattle over 4 years old were reactors (Table 3).

Comparison of hematological status and serological results among 615 beef cattle is given in Table 4. Twenty-five (4.1%) ani-

mals showed lymphocytosis and 82.0% of all the animals was hematologically normal according to the key for Japanese Black cattle [4]. Most (88.0%) of the cattle which showed lymphocytosis were positive for BLV antibodies. However, 38.3% of the normal animals also had antibodies against BLV.

Discussion

During a 10-year period from 1969 to 1978, a clinical diagnosis of bovine leukosis was made on 45 Japanese Black cattle in the Hida district. Seventeen (37.8%) of the 45 cattle were from T village. The average incidence of bovine leukosis was 38 per one million cattle in the Hida district and 246 in T village. Although the number of beef cattle kept in T village was limited, the incidence of bovine leukosis was surprisingly high in this village, as compared with that in the United States, Denmark, and even in other districts of Japan. It was 5.5 per one million dairy cattle and 0.29 per one million beef cattle in California [13]. In an infected area of Denmark it was about 29 and 10 per one million cattle in 1960 and 1963, respectively [1]. In beef cattle (Japanese Shorthorns) in the Towada district of Japan, the average incidence was 165.5 per one million cattle [15].

Cattle from A, Ku and T villages, where they were put together on the same pasture during summer (conventional style), showed higher rates of reactors (20.0 to 59.2%, averaging 52.1%) than those from other villages (0 to 12.5%, averaging 8.1%), where they were kept in a single herd throughout the year (modern style). The difference in rate of reactors may have been caused by the difference in herd style. The cattle from herds of conventional style had a chance to contact infected cattle of other farmers. They may have exposed more frequently to transmissible BLV than the cattle from

herds of modern style [8, 11]. Therefore, the difference in rate of reactors between the two herd styles may have been caused by the difference in frequency of contact with infected cattle between these styles.

An astounding rate, or 59.2%, of the beef cattle in T village was found to be positive for BLV antibodies, especially 70.9% of the cattle in area d of the village. These results showed a more widespread occurrence of BLV infection in T village than in the Towada district, where multiple leukosis has been reported [15]. The Japanese Black cattle in each area of T village have mainly been bred and raised with relatively little interchange with cattle from any other area of the village, especially those from area d. Since most of the dams in the village are positive for BLV antibodies, the congenital transmission of BLV to offspring may be one of the reasons for having formed such highly enzootic area [8]. No exact reasons are known as yet.

Eighty-eight percent of the animals which had shown lymphocytosis in the hematological test had antibodies against BLV, whereas 38.3% of the normal animals studied also had these antibodies. Therefore, it was indicated that there was a relationship between BLV infection and the level of lymphocytosis, as is described previously [5, 6]. It was, however, observed that 276 (44.9%) of 615 beef cattle had antibodies against BLV, and that only 25 (4.1%) of them showed lymphocytosis. This observation suggests that the serological reaction may appear earlier than hematological disorders. The hematological test, however, is much superior to the serological test in detecting the onset of the clinical sign of the disease, as described previously by the authors [3].

The difference in percentage of reactors in cattle between the two different herd

styles suggests that the control and eventual eradication of bovine leukemia from a herd may be accomplished by the separation of infected cattle from normal animals. Since infection was suggested to be transmitted from dam to fetus in a previous paper [8], the slaughter of infected dams may be considered desirable.

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要 約

黒毛和種における牛白血病ウイルスの血清疫学調査：小沼 操・石原勝也*・大谷 健**・本間利夫・見上 彪・伊沢久夫(北海道大学家畜伝染病学教室，*岐阜大学家畜内科学教室，**飛騨家畜保健衛生所)——牛白血病ウイルス (BLV) の糖タンパク抗原を用いたゲル内沈降反応により，岐阜県飛騨地方の黒毛和種の血清疫学調査を行なった。調査対象となった黒毛和種は2つの異なる方法で飼育されており，1つは各農家のウシを集めて夏のあいだ山地に放牧するもので，他の1つは年間を通じて農家ごとに各自飼育するものである。夏のあいだ山地に放牧したウシでは，その半数以上 (52.1%) が BLV 抗体を保有していた。これに対して，各農家で飼育したウシでは少数 (8.1%) のみが BLV 抗体をもつにすぎなかった，この成績は，飼育形態によって BLV 抗体保有率に差が生ずることを示すものと思われる。なお，被検牛の抗体陽性率はウシの加齢とともに増加した。いっぽう，血液検査によりリンパ球増加症と判定されたウシの 88% が，また血液検査で正常と判定されたウシの 38.3% がそれぞれ BLV 抗体を保有していた。この所見は，BLV 感染とリンパ球数の増加の間には関連のあることを示すものと思われる。