Survey for Antibodies to Bovine Leukemia Virus in Dairy and Beef Cattle in Japan

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(Received for publication March 13, 1978)

Abstract. A seroepidemiological survey for antibodies to bovine leukemia virus (BLV) in dairy and beef cattle in Hokkaido and in the Towada district was performed using BLV glycoprotein antigens by the immunodiffusion test. Ninety-four of 2878 sera (3.3%) from the 14 districts of Hokkaido were positive for BLV antibodies. In contrast, in the Towada district, where enzootic bovine leukosis has been previously reported, 363 of 1127 sera (32.2%) were reactive. The number of reactive samples varied from 6.9 to 52.7% in 13 pastures in the Towada district and the percentages increased gradually with age. No significant difference in lymphocyte counts between BLV antibody positive and negative beef cattle was found. Thirty sera from cattle with naturally occurring lymphosarcoma were also tested. The sera from the adult form of bovine lymphosarcoma reacted with BLV glycoprotein antigens, but the sera from the other forms of bovine lymphosarcoma did not.

Bovine lymphosarcoma is a neoplastic disease of the lymphoid tissue. The four forms of bovine lymphosarcoma are clinically distinct from each other [2, 22]. The most common adult form is a contagious disease induced by bovine leukemia virus (BLV) [9]. Several serological methods have been designed and used for diagnosis of BLV infection. These methods include an immunodiffusion (ID) test using BLV p24 (p24-ID) [10] or glycoprotein antigens [19, 20], a complement-fixation (CF) test [11], an immunofluorescence (IF) test [6] and a radioimmunoassay (RIA) using BLV p24 (p24-RIA) [4].

Recent comparative studies of serological

tests revealed that the ID test using BLV glycoprotein antigens (gp-ID) was the best test for diagnosis of BLV infection [1, 14].

Several cases of enzootic and sporadic bovine leukosis have been observed in Japan. However, the frequency of BLV antibody-positive cattle among clinically normal cattle is still unknown. This is the first survey for antibodies to BLV glycoprotein (gp) antigen in Japan. We will describe a preliminary local survey for BLV antibodies in beef and dairy cattle in the Towada district and in Hokkaido.

Materials and Methods

Sera: Thirty sera from cattle with naturally occurring lymphosarcoma were obtained from Drs.

日本の乳牛および肉牛における牛白血病ウイルスの抗体調査: 小沼 操・本間利夫・見上 彪 (北海道大学獣医学部家畜伝染病学教室), 吉川博康・吉川 堯 (北里大学獣医畜産学部病理学教室)

K. Ooshima (Iwate Univ., Morioka, Iwate), S. Ichijo, (Obihiro Univ. of Agriculture and Vet. Med., Obihiro, Hokkaido) and M. Sonoda (College of Dairy Agriculture, Ebetsu, Hokkaido). Lymphosarcoma was diagnosed and classified into four forms by both clinical and pathological observations.

The present seroepidemiological survey for BLV antibody detection was carried out on sera from dairy and Japanese beef cattle. Sera from 2878 cattle (mainly dairy cattle) were collected randomly from 1974 to 1975 at a rate of about 0.4% from all cattle in Hokkaido. These sera were obtained from the fourteen Livestock Hygiene Service Centers (Fig. 1), where they were collected for the detection of brucellosis.

Sera from 1127 Japanese beef cattle (Japanese black hair and Japanese short horn) in 13 pastures were collected in 1977 in the Towada district in Aomori Prefecture as shown in Fig. 2.

The standard anti-BLV serum which obtained from a sheep (V34) [19] experimentally infected with BLV was used as a positive serum control in both ID and CF tests.

Antigen preparation: The antigen for the ID test was prepared from culture fluids from a BLV-infected cell line (A-77thv+) [20] as previously described [19, 20]. Briefly, culture fluids were centrifuged at 66,000 g for 90 min, and the supernatant was concentrated 100 to 200 times by dialysis against polyethylene glycol (#6,000). This concentrated preparation contained both gp and p24 antigens [19]. Either this concentrated preparation or gp antigens which were further purified by Concanavalin A-Sepharose [20] were used as antigen. The antigen for the CF test was prepared from culture fluids from the same cell line. This antigen was concentrated about five times to obtain 4 antigenic units using V34 serum.

Immunodiffusion test: The ID test for detection of antibodies to gp antigens of BLV was performed as previously described [15, 19] with slight modification. In this experiment, ID tests were run with 1% agarose prepared with 0.05M Tris-HCl buffer (pH 7.2) containing 8.5% NaCl. A satisfactory method for detection of precipitating antibodies against gp antigens has been to use a central 4 mm antigen well separated 3.5 mm from six peripheral 4 mm serum wells. A positive serum obtained from cattle or sheep with lymphosarcoma was placed in two peripheral wells as control. Wells were filled only once and incubated at room temperature in a humidified chamber. After 72 hours of incubation, the results were read.

Complement fixation test: The CF test was conducted with guinea pig complement including 5%

unheated normal calf serum and sheep red blood cells sensitized with rabbit hemolysin as previously described [11]. The CF titers of V34 sheep serum using purified gp and p24 antigens were 1 : 128 and 1 : 32, respectively.

Results

Sera from thirty cattle with naturally occurring lymphosarcoma including 21 with the adult form, one with the calf form, 6 with the thymic form and two with the skin form of lymphosarcoma were tested for BLV antibodies by the ID and the CF tests. Seventeen of the 21 sera from cattle with the adult form were positive by the gp-ID test, and eighteen sera were positive by the CF test. However, all 9 sera from cattle with calf, thymic and skin forms of lymphosarcoma were negative using both the gp-ID and the CF tests (Table 1).

Bovine leukosis occurs only sporadically in Hokkaido. Results of the survey for antibodies to BLV gp antigens using the

Table 1. Demonstration of antibody in cattle with lymphosarcoma

Origin of serum	gp-ID	CF
Adult form	17/21 ^{a)}	18/21
Calf form	0/1	0/1
Thymic form	0/6	0/6
Skin form	0/2	0/2

Remarks.

a) : Values are number of cattle with antibody against BLV per total number of cattle tested.

Fig. 1. Map of Hokkaido showing the 14 districts

2878 sera from the 14 districts of Hokkaido are shown in Figure 1. Ninety-four (3.3%) were positive for antibodies, and reactors

Table 2. Reactors to the precipitin test for antibody to BLV antigens in sera of cattle from Hokkaido

District	No. of sera tested	No. of reactive sera	%
Oshima	149	9	6.0
Hiyama	97	2	2.1
Shiribeshi	68	0	0
Iburi	141	0	0
Ishikari	479	1	0.2
Sorachi	98	5	5.1
Rumoi	150	4	2.7
Kamikawa	134	4	3.0
Sohya	292	0	0
Hidaka	150	4	2.7
Tokachi	138	9	6.5
Abashiri	433	19	4.4
Kushiro	299	21	7.0
Nemuro	250	16	6.4
Total	2878	94	3.3

Fig. 2. Outline map of Towada district in Aomori Prefecture showing the location of the 13 pastures sampled

varied from 0 to 7% among the 14 districts of Hokkaido as shown in Table 2. The numbers of reactors were relatively low in cattle from the northern and central districts of Hokkaido (Ishikari, Iburi, Shiribeshi, Sohya, Hiyama, Rumoi and Hidaka) compared with cattle from the eastern districts (Kushiro, Abashiri, Tokachi and Nemuro). There were also relatively fewer reactors (0.5 to 1.5%) in cattle less than 2 years old than in cattle 2 years or older (2.5 to 3.5%).

A similar antibody survey was performed in the Towada district where enzootic bovine leukosis has been previously reported [24]. Eleven hundred and twenty-seven sera from beef cattle in the Towada district were tested, and 363 sera (32.2%) were reactive. The reactors varied from 6.9 to 52.7% among the different pastures as shown in Table 3. When these results are grouped according to age, no reactors were found in cattle less than 2 years old, and the number of reactors gradually increased with age. One hundred and forty-six cattle of the 354 cattle (41.2%) over 8 years old were reactors (Table 4).

Table 3. Reactors to the precipitin test for antibodies to BLV antigens in sera of cattle in the Towada district

Pasture	No. of sera tested	No. of reactive sera	%
A	74	39	52.7
B	30	4	13.3
C	30	8	26.7
D	50	17	34.0
E	34	13	38.2
F	192	71	40.0
G	30	3	10.0
H	58	4	6.9
I	92	14	15.2
J	116	41	35.3
K	185	71	38.4
L	35	10	28.6
M	201	68	33.8
Total	1127	363	32.2

Table 4. Reactors to the precipitin test for antibodies to BLV antigens in sera of beef cattle from the Towada district grouped according to age

Age (years)	gp-ID	%
1—2	0/9 ^{a)}	0
2—4	23/146	15.8
4—6	68/275	24.7
6—8	88/219	40.2
>8	146/354	41.2
Total	325/1003	32.4

Remarks.

a) : Reactors/No. tested.

Table 5. Lack of relationship between lymphocyte counts and gp-ID results in 876 Japanese short horn cattle

Age (years)	Lymphocyte counts	
	gp-ID test negative	gp-ID test positive
2—3	6763±2161 ^{a)}	6340±1184
3—4	6046±2245	6083±2118
4—8	5938±3088	6625±3308
>8	5167±2403	5156±2102

Remarks.

a) : Mean value ± standard deviation.

The lymphocyte counts of antibody-positive cattle were compared with those of antibody-negative cattle in the Towada district. There was no significant difference in lymphocyte counts between the BLV antibody-positive and the BLV antibody-negative cattle from 876 Japanese beef cattle (Table 5).

Discussion

Until BLV was isolated from bovine lymphosarcoma material, the diagnosis of bovine leukosis had been conducted by hematological tests [2, 23]. Recent progress in BLV research has made it possible for diagnosis of bovine leukosis by serological methods. Serologically detectable antibody has been regularly found in the sera of leukotic animals and in animals of multiple herds with a high frequency. Antibody to

BLV has also been detected in clinically and hematologically normal cattle [5, 8]. In the present experiments, the absolute lymphocyte counts from cattle with and without antibody to BLV were almost identical. Therefore, serological methods seem to be a more accurate means of detection of BLV-infected animals and could efficiently replace the hematological methods which were used for eradication of the disease.

Three serological methods, ID, IF and CF, have mainly been used for demonstrating antibody to BLV. Initially, the ID test was designed to identify an antibody to a major polypeptide (p24) obtained by destruction of purified BLV by ether [10]. This test was also very useful for early epidemiological studies of BLV infection in various cattle populations. However, comparative studies with CF and IF tests clearly showed that some BLV-infected cattle were not detected by the p24-ID test [5]. Recently, Onuma et al. [19] described a second BLV-associated antigen which was present in the culture fluid from infected cells. This antigen, which was ether-sensitive and a glycoprotein in nature, was purified by Concanavalin A-Sepharose [20], and it was found to be located on a glycoprotein of the BLV envelope [16]. The gp-ID test has been found to be equally or more sensitive than the CF test [12, 19]. Using BLV-infected culture fluid as antigen, the CF test can measure antibodies to gp and p24 whereas the ID test can demonstrate antibody to either p24 or gp depending on the specific antigens used. The results obtained by CF and gp-ID tests were almost same in sero-epidemiological studies [12, 13, 19], indicating that the major reaction detected by the CF test may be due to gp antigen and antibody complex. Comparisons of the serological methods (p24-ID, CF, IF, gp-ID

and p24-RIA) for detection of BLV antibodies in reference sera resulted in a good agreement among various laboratories except that p24-ID was found to be less sensitive [14]. Therefore, to detect antibodies to BLV, the gp-ID test has been recommended as the method of first choice by the International Meeting on Bovine Leukosis because it is specific, highly sensitive and extremely simple to perform [1].

Among the four forms of bovine lymphosarcoma, sera from only the adult form reacted to both the gp-ID and CF tests. Similar observations using the gp-ID and the p24-RIA tests have been reported [4, 19]. In cases of the adult form, BLV was demonstrated in cultured cells from affected organs [17] and the BLV genome was also detected in the lymphosarcoma cells [3, 7] but not in the other forms. These observations may suggest that the calf, thymic and skin forms are not etiologically related to the adult form which is associated with BLV.

The results of our antibody survey for BLV in cattle of Hokkaido and the Towada district have indicated more wide-spread occurrence of BLV infection in the Towada district than in Hokkaido. In the Towada district but not in Hokkaido, multiple leukosis has been reported [24]. Most of the beef cattle in the Towada district are together in the pasture during May to October. Since BLV is mainly transmitted horizontally by contact among cattle [21], this may explain the wide-spread occurrence of BLV infection in the Towada district. In contrast, most dairy cattle in Hokkaido are kept in barns, and there is less contact among cattle. Although a high percentage of reactors was found in the Towada district, only few cases of bovine lymphosarcoma (165 per 100,000 cattle) had been reported from 1968 to 1971 [24]. This sug-

gests that not all cattle infected with BLV develop lymphosarcoma. The development of a new diagnostic test to detect reactors which will develop lymphosarcoma in future is needed.

Recently Onuma and Olson [18] reported a tumor-associated antigen on bovine lymphosarcoma cells. The same antigen was also detected on the peripheral blood lymphocytes (PBL) from BLV-infected cattle with no evidence of lymphosarcoma. Detection of this kind of antigen on PBL from BLV-infected cattle may help in the development of a new diagnostic test. To eradicate bovine lymphosarcoma, it will be necessary to detect lymphosarcoma as well as BLV infection in cattle and to separate these animals from negative animals.

Acknowledgments: We wish to express our appreciation to Drs. H. Satoh (Ishikari Livestock Hygiene Service, Hokkaido) and S. Yoshida (Tokachi Livestock Hygiene Service, Hokkaido) for kind suggestions. We acknowledge Miss Y. Matsuzawa for preparation manuscript and for technical assistance.

This work was supported in part by Grant-in-Aid (No. 257133) from The Ministry of Education, Science and Culture, Japan.

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