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Citation	Japanese Journal of Veterinary Research, 40(2-3), 105-111
Issue Date	1992-09-30
DOI	https://doi.org/10.14943/jjvr.40.2-3.105
Doc URL	https://hdl.handle.net/2115/2402
Type	departmental bulletin paper
File Information	KJ00002377601.pdf



ATTEMPT TO ERADICATE BOVINE LEUKEMIA VIRUS-INFECTED CATTLE FROM HERDS

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(Accepted for publication : August 12, 1992)

Key Words : Bovine leukemia virus, bovine leukosis, eradication.

The bovine leukemia virus (BLV) is accepted universally as the causative agent of the enzootic form of bovine lymphosarcoma, a fatal neoplastic disease, and persistent lymphocytosis, usually a benign condition that is common in BLV-infected herds²⁾. Although BLV is transmitted both vertically and horizontally^{3,11)}, the latter is the predominant mode in field conditions^{1,12)}. Evidence implicates the involvement of a blood-sucking vector^{1,14)}. Since practically all BLV-infected cattle possess a specific antibody to the virion glycoprotein antigen of the virus, several serological methods have been described for the detection of BLV antibodies in sera, such as the agar gel immunodiffusion (AGID) test^{9,12,20)} and enzyme-linked immunosorbent assay (ELISA)^{5,18,21)}. Immunologically, it was reported that the complement-dependent antibody cytotoxicity (CDAC) test for peripheral blood lymphocytes using monoclonal antibodies against tumor-associated antigen (TAA) might be useful in the diagnosis of enzootic bovine lymphosarcoma¹³⁾. The most important principle in an eradication program is the elimination or segregation of infected cattle^{4,19)}. Successful results using this idea have been reported^{6,10,15,17,22)}.

In this report, we describe how the segregation or elimination method successfully results in eradication of BLV-infected cattle in farms. We studied herds in two different environments : Dairy farm A is a farm of 27.1 hectares located in southern Taiwan. The farm neighbors agricultural fields and is 6 km away from the nearest dairy farm. The herd numbered approximately 400 in February 1984. In March of the same year, 166 pregnant cows were imported from the U. S. A. The farm raised their cattle separately in blocks. The milk herd was kept in a tie-stall barn before 1986, after which it was moved to a newly constructed free-stall barn, which was 120 m from the heifer herd. The calf herd was situated 25 m from the heifer herd and 120 m away from the milk herd. All cows, BLV antibody-positive and -negative, in

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various pregnancy and labor phases were kept in the same barn, but new born calves were promptly moved to a separate barn and fed artificially with colostrum and milk until weaning, then moved in with the heifer herd 6 months later. Serologic surveys of the herd were conducted for antibodies to BLV by the AGID test and ELISA combined method^{20,21)} with 9 serial tests between May 1987 and October 1990. For the first 4 tests the AGID test was used at 3- or 6-month intervals. We tested the herd at 2-4 months intervals using the AGID and ELISA combined method. All cattle seven months of age were subjected to the test and those found positive were either isolated at a location 6 km away or slaughtered.

Farm B is located in the suburbs of Sapporo, Japan. The farm had kept approximately 40 bulls, aged 4-5 years for taking semen. Antibodies to BLV were checked 2 to 3 times annually by the AGID test. In August 1985 and April 1986, TAA was detected in peripheral blood lymphocytes (PBL) by the CDAC test using monoclonal antibody c143¹³⁾.

Cattle of dairy farm A showed positive to antibodies against BLV at the rates of 30.2%, 30.4%, 23.5%, 5.2%, 2.7%, 0.9%, 0.8%, and 0% for the first 8 tests, respectively. Some of the BLV-positive cattle were segregated from the herd (total 103 cattle) during the first 4 tests (May 1987-May 1988). These procedures reduced the infection rate from 30.2% to 5.2%. After November 1988, we decided to eliminate all BLV-positive cattle from the herd and a total of 12 positive cattle during the period between November 1988 and November 1989 were segregated and later slaughtered. After May 1988, only BLV-negative animals were newly introduced. As a result of these segregation and quarantine procedures, no positive cattle have been detected since November 1989 (Table 1).

In farm B, the first AGID test was done in 1980 and the positive rate was 13.8%. Since BLV-positive animals increased to about 30% in 1984 and 1985, we planned to eradicate the positive animals from the farm. Thus we started to check the newly introduced animals and only BLV-negative animals were introduced after August 1985. In August 1985, 11 of the 36 bulls were BLV-antibody positive. All of the positive animals were also tested for the expression of TAA in their PBL and 6 of the 11 were found to be TAA-positive. Previously, we found that BLV-infected and TAA-positive animals had a higher potential for developing tumors in the future when compared to BLV-infected but TAA-negative animals¹³⁾. Thus, only these 6 TAA-positive bulls were eliminated from the farm for slaughter, and the other 5 BLV-infected but TAA-negative bulls were kept in the farm because of the economical reasons. In April 1986, the previously noted 5 BLV-infected animals plus 3 more animals were BLV-positive. These 3 converted to positive during the tests. All of these 8 BLV-infected bulls were TAA-negative; however, we decided to segregate all of these 8 bulls to completely eradicate BLV-infected animals from the farm. These 8 BLV-positive bulls had been kept in an isolated barn which was located about 1500 m from

Table 1. Eradication of BLV-infected cattle from A dairy farm

Test	Time tested	Presence of Ab No. pos./No. tested(%)	No. eliminated by the next test(No. Ab ⁺ cattle)	No. newly introduced by the next test (No. Ab ⁺ cattle)	Positive conversion ^{c)}
1	1987 May	89/294 (30.2)	41 (14) ^{a)}	0(0)	2/253
2	1987 November	77/253 (30.4)	13(13)	100(2)	14/340
3	1988 February	80/340 (23.5)	65(62)	65(0)	0/340
4	1988 May	18/340 (5.2)	23(14)	13(4)	1/330
5	1988 November	9/330 (2.7)	6(6) ^{b)}	6(0)	0/330
6	1989 May	3/330 (0.9)	3(3)	23(0)	0/350
7	1989 July	3/350 (0.8)	3(3)	3(0)	0/350
8	1989 November	0/350 (0)	0(0)	30(0)	0/380
9	1990 October	0/380 (0)	0(0)	0(0)	0/380

^{a)} Antibodies to BLV were detected by the AGID test. Ab⁺=Antibody positive.

^{b)} Antibodies to BLV were detected by the AGID test and ELISA combined method.

^{c)} No. positive in the next test/Total No. of sera tested in the next test which were negative in the previous test.

the farm. In July 1986, all of the 34 bulls were BLV-negative. Since then no positive animals have been detected on this farm (Table 2).

The results suggest that complete decontamination of herds from BLV infection can be accomplished by eradicating positive animals with AGID test. Straub¹⁷⁾ achieved complete decontamination after five serial tests, Yoshikawa et al²²⁾ after three serial tests, and Ohshima et al¹⁵⁾ eradicated BLV infection after seven serial tests. We succeeded in completely eradicating BLV infection in herds A and B after seven and four serial tests, respectively.

The AGID test is the simplest and the most common, but the test shows substantially less sensitivity than ELISA, particularly for the detection of BLV-infection in the early stages and during parturition stages^{5,18,21)}. For example, during the 6th test at the dairy farm A, we discovered that one parturition cow and one heifer in its early stage of infection showed positive with the ELISA test but negative with the AGID test.

We have shown that the imported cattle introduced BLV infection at dairy farm A²⁰⁾. We found in the 2nd test that some seropositive calves were born from negative dams. Horizontal transmission is the most likely mode of BLV transmission through intradelivery barn contact^{7,16)} or blood-sucking insects. Tabanid flies or biting flies appear on occasion during the summer. The role of tabanid flies in the spread of BLV has been suggested^{1,14)}, but it has been ignored in Taiwan.

In summary, the results of this study showed that early segregation or elimination of infected cattle is effective for eradication of leukemic herds. Particularly in Taiwan, where the free-stall farm style has been the main dairy farming technique, we strongly recommend such segregation and elimination measures.

Table 2. Eradication of BLV-infected cattle from B farm

Time tested	Presence of Ab ^{a)} No. pos./No. tested(%)	No. eliminated by the next test(No. Ab ⁺ cattle)	No. newly introduced by the next test (No. Ab ⁺ cattle)	Positive conversion ^{d)}
1980 April	5/36(13.8)	22 (1)	24(4)	4/38
1984 May	12/38(31.6)	7(3)	5(2)	0/36
1985 August	11/36(30.6)	6(6) ^{b)}	11(0)	3/41
1986 April	8/41(19.5)	8(8) ^{c)}	1(0)	0/34
1986 July	0/34(0)	6(0) ^{e)}	11(0)	0/39
1986 December	0/39(0)	2(0) ^{e)}	3(0)	0/40
1987 March	0/40(0)	7(0) ^{e)}	11(0)	0/44
1987 July	0/44(0)	4(0) ^{e)}	4(0)	0/44
1987 December	0/44(0)			

a) Antibodies to BLV were detected by the AGID test. Ab⁺ = Antibody positive.

b) Six of 11 BLV-positive cattle were TAA-positive in their PBL by the CDAC test.

Thus these 6 were eliminated from the farm and the other 5 were still kept in the farm.

c) All of the 8 BLV-positive but TAA-negative bulls were segregated from the farm.

d) No. positive in the next test/Total No. of sera tested in the next test which were negative in the previous test.

e) The bulls were eliminated only for economical reasons.

ACKNOWLEDGEMENTS

This study was supported by Council of Agriculture, Taiwan, Republic of China. We thank Prof. S. Namioka of the Faculty of Veterinary Medicine, Hokkaido University, Japan: Prof. C. H. Chang of the Department of Veterinary Medicine, National Taiwan University: and Dr. T. C. Lin, Chief of the Animal Health Division, Council of Agriculture, Taiwan, for their encouragement. We also wish to express our appreciation to the Livestock Disease Control Center in Taiwan for collecting serum samples.

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