Serological Methods to Detect Calves Infected in Utero with Bovine Leukemia Virus

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(Received 28 January 1983/Accepted 6 May 1983)

ABSTRACT. Calves born to dams infected with bovine leukemia virus (BLV) were investigated serologically and virologically before and/or after ingestion of colostrum to develop effective methods to detect BLV-infected calves in an early postnatal life. Of 50 precolostral sera, 10 (20%) and 13 (26%) were BLV-antibody positive by immunodiffusion (ID) and virus-neutralization (VN) tests, respectively, indicating in utero BLV infection of the donor calves. In 18 postcolostral sera, ID antibody titers against BLV correlated well with serum ID antibody titers of the respective dams. The colostral antibodies decayed with a half-life of about 20 days in 76 BLV-uninfected calves. The antibody became undetectable in almost all of the calves at 6 months of age. On the other hand, in 16 calves infected in utero with BLV, ID antibody was often detected even at 6 months of age and later. The pattern of change in individual sequential ID antibody titers of these BLV-infected calves could be divided into three types. In type 1, ID antibody titers were maintained from birth to 7 months of age or older. In Types 2 and 3, the antibody titers decreased at a rate similar to those in uninfected calves. The titers then increased again at 5 to 7 months of age in Type 2 but they did not appear again after their disappearance in Type 3. Calves with antibody changes of Types 1, 2 and 3 were 6 (37.5%), 6 (37.5%) and 4 (25.0%), respectively. By testing sequential antibody titers, it was revealed that 7 (11.7%) of 60 calves which could not be tested for precolostral antibodies showed antibody changes of Types 1 and 2, indicating BLV infection in utero. Methods to detect BLV-infected calves in an early postnatal life are discussed.

Bovine leukemia virus (BLV) infects cattle persistently for life [5]. Therefore, it has been shown that separation of BLV-infected cattle from uninfected cattle is the most effective method for eradication of BLV infection (14, 20, 22, 23). BLV is transmitted horizontally and vertically as well as other leukemia viruses [1, 18]. Horizontal transmission of the virus among cattle can be demonstrated with confidence by serological tests [15]. In utero transmission also can be demonstrated by testing precolostral and fetal sera for BLV antibodies [17, 18], since the bovine fetus is capable of humoral antibody production after the

third month of gestation [19]. This method, however, is not a practical one since collection of precolostral serum is fairly difficult. After ingestion of colostrum serologic diagnosis of BLV cannot be performed during the period of persistence of precolostral antibody because there is no effective method for distinguishing between BLV-induced active antibodies and passive colostral antibodies.

In this study, the antibody decay rates in infected and uninfected calves were compared to assess in utero transmission of BLV in early postnatal life. The rate of in utero transmission of the virus was

also studied by using precolostral sera and sequentially collected sera.

MATERIALS AND METHODS

Animals: Cattle in four herds were observed. The prevalence of antibody to BLV in these herds is shown in Table 1. Serum samples were obtained from calves born to seropositive dams before they received colostrum and/or a few days after birth and then serially at appropriate intervals. These sera were stored at -20°C until use.

Calves which had no antibody detectable by either the virus-neutralization (VN) or immunodiffusion (ID) test at birth and which had no detectable BLV antibodies for at least 3 months after the last detectable colostrum antibody were assumed to be uninfected with BLV. On the other hand, calves which has antibodies at birth and/or calves from which BLV was isolated until 50 days of age were assumed to be infected with BLV in utero.

Virus isolation: Virus isolation was performed by the syncytium induction assay as described previously [4, 6] with slight modification. Fourth to seventh passages of bovine embryonic spleen (BESP) cells were used as indicator cells. Briefly, BESP cells in amounts of 3.5×10^4 suspended in 0.5 ml of medium consisting of Eagle's minimal essential medium containing 10% fetal calf serum were seeded in wells of a multi-dish tray (Linbro, FB-16-24-TC) and incubated at 37°C . One day later, the culture was inoculated with 2×10^5 lymphocytes of the animal to be tested for BLV infection. Then, to confirm the specificity of the syncytia, the culture were incubated with culture medium containing anti-BLV serum but not anti-bovine syncytial virus serum or anti-bovine syncytial virus serum but not anti-BLV serum. The cultures were stained

and syncytia were counted 7 days after inoculation. All lymphocyte samples and appropriate controls were assayed in duplicate.

VN test: A syncytium inhibition test was used for detection of neutralizing antibody to cell-free virus [4, 6]. Serum heated at 56°C for 30 minutes at a dilution of 1:4 was mixed with an equal volume of viral suspension and the mixture was incubated at 37°C for 1 hour. Then, 0.1 ml of the above mixture containing $2 \mu\text{g}$ of polybrene per ml was inoculated into a BESP cell culture. After the culture were incubated at 37°C for 1 hour in a CO_2 incubator, 0.4 ml of the medium that was described in the previous section and contained $2 \mu\text{g}$ of polybrene per ml was added and the cultures were incubated for 7 days. The cultures were then stained and syncytia were counted. All test samples and virus controls were assayed in duplicate. In this test, sera which give 85% or more, 65 to 84% and less than 65% reduction in syncytium formation as compared with the control cultures are considered positive, equivocal and negative, respectively.

ID test: The test for antibody against glycoprotein antigen of BLV was as described previously [10]. The antibody titer is expressed as the reciprocal of the highest serum dilution that showed a positive reaction.

RESULTS

1. BLV antibodies in precolostral sera.

Fifty calves born to seropositive dams in four herds were tested for BLV antibodies with precolostral sera. Ten (20%) and 13 (26%) of them were positive for BLV antibodies by ID and VN tests, respectively (Table 1). All of the ID antibody-positive sera were positive by the VN test. The prevalence of precol-

Table 1. Prevalence of precolostral antibody to BLV in calves in four herds

Herd	No. of calves tested	Antibody at birth*	
		ID (%)	VN (%)
A (65.0%)**	17	6 (35.3)	8 (47.1)
B (28.2%)	7	2 (28.6)	2 (28.6)
C (29.0%)	12	0 (0)	1 (8.3)
D (43.8%)	14	2 (14.3)	2 (14.3)
Total	50	10 (20.0)	13 (26.0)

* ID: Immunodiffusion; VN: Virus-neutralizing.

** BLV-antibody positive rate of the herd.

ostrual antibody-positive calves was markedly different in each herd, with a range of 8.3% to 47.1%.

2. Antibody titers after ingestion of colostrum

Eighteen pairs of sera collected from dams and their calves at birth or 1 or 2 days after calving were compared by the ID test (Fig. 1). There was a high correlation between the two ($r=0.69$). However, the average serum antibody titer (12.1) of the calves was higher than that (7.4) of the dams.

Decay of colostral antibodies in uninfected in uninfected calves was tested by using 218 sera collected serially from 76 calves (Fig. 2). Ten and 20 of them were tested for BLV by syncytium induction assay during the precolostral antibody-positive period (10–100 days of age) and after disappearance of the antibody (129–310 days of age), respectively, and proved to be free of BLV. All the calves had detectable antibodies after ingestion of colostrum. The antibody titers averaged about 8 at 1 month of age. The antibody titers decreased exponentially to undetectable levels thereafter. The half-life of the antibodies was estimated to be roughly 20 days. The prevalence of antibody-positive calves decreased with increase in age to 50 to 60%

Fig. 1. Correlation between antibody titers of dams and those of calves.

and about 6% at 3 and 5 months, respectively. There was no calf that had an antibody titer higher than 1 at 5 months. No calves had apparent ID antibody at 6 months.

More than half of the uninfected calves were also examined by VN (Fig. 3). At 1 month of age all the calves were positive. The prevalence of positive individuals decreased to about 50% by 4 months. Most of the positive calves changed to equivocal reactors by 5 or 6 months of age and became negative reactors after 7 months.

Change in antibody titers in infected calves were investigated serially for 11 precolostral antibody-positive calves and five BLV-positive calves. As shown in Fig 4, there were many calves that had positive antibody titers even at 6 months of age or older.

Although sequential changes in antibody titers differed with the individual animal, they could be divided into three groups. Three typical examples of each group are shown in Fig. 5. An increase in antibody titers was observed after ingestion of colostrum in all calves that had

Fig. 2. Decay of colostral immunodiffusion antibody titers to BLV based on 218 observations representing repeated samples from 76 calves believed not to be infected with BLV.

○: Calves tested for BLV infectivity. All the calves were negative.

*: Percentage of calves with colostral antibody to BLV at the indicated age.

** : Precolostral sera. 33 samples were tested.

Fig. 3. Decay of colostral neutralizing antibody to BLV based on 168 observations representing repeated samples from 35 calves believed not to be infected with BLV.

*: +, ± and - indicate sera which give 85% or more, 65 to 84% and less than 65% reduction, respectively, in syncytia formation as compared with the control cultures.

** : Precolostral sera.

Fig. 4. Changes in immunodiffusion antibody titers to BLV based on 74 observations representing repeated samples from 16 calves believed to be infected with BLV.

*(○): Calves tested for BLV infectivity. All the calves were positive.

** : These sera were negative by immunodiffusion but positive by virus neutralization.

***: Precolostral sera.

low titers of precolostral antibodies. In the first group (Type 1) precolostral and/or colostral antibody titers were maintained at the primary level, increased a little or decreased gradually until 7 to 10 months of age. This type was observed in six calves (37.6%). In the second group (Type 2), ID antibody titers decreased to undetectable levels at 3 to 6 months of age with an antibody decay rate similar to that of uninfected calves. VN antibody, however, was positive at the ID antibody-negative stage. Following this stage that antibody titer increased again. This type was also observed in six calves (37.5%). In the third group (Type 3), after ID antibody disappeared in the same way as in the calves of the second group, the antibody remained negative until the end of the observation period of 8 to 10 months. This type was observed in four calves

(25.0%). In this type, all the calves changed to VN antibody-negative after the disappearance of ID antibody. Two calves, however, developed VN antibody 2 and 3 months after the disappearance of the preexisting VN antibody. The remaining two calves remained negative for VN antibody until the end of the observation period.

The relationship between the existence of precolostral antibody and changes in antibody titers after ingestion of colostrum was investigated (Table 2). All calves without ID and VN antibodies at birth showed antibody decay of Type 3. Calves with precolostral VN antibody but no ID antibody showed decay of Type 2 or 3. Calves with precolostral ID and VN antibodies showed decay of Type 1, 2 or 3.

3. Detection of BLV-infected calves by serial antibody tests.

Fig. 5. Individual sequential changes in immunodiffusion antibody titers to BLV in calves believed to be infected with BLV. Three calves representing characteristic antibody-titer changes of each type are shown.

*: These two calves were negative by immunodiffusion but positive by virus neutralization.

Because the test for sequential antibody titers for BLV is considered to be a useful method for detecting BLV-infected animals in the early postnatal period, 60 calves born to seropositive dams were tested. Three serum samples were collected from each calf at intervals of 1 to 2 months and tested for antibody titers. Seven (11.7%) of them showed antibody changes of Type 1 or 2, suggesting in utero BLV infection. BLV was isolated from five of the seven calves at 30 to 50 days of age. The other two calves were not tested for BLV infectivity.

DISCUSSION

Detection of precolostral antibody to BLV is probably the most effective method for detecting calves infected in utero [18]. The collection of precolostral serum, however, is very painful and it is almost impossible to use it for all calves. Therefore, the possibility of detecting infected calves by testing for changes in sequential antibody titers was investigated.

The colostral antibody titers decreased with a half-life of about 20 days, the same as for antibodies against other infectious agents in cattle [2, 11, 13]. The number of calves having colostral antibodies against BLV decreased to 6% and 0% by

Table 2. Relationship between antibody at birth and types of antibody-titer changes after ingestion of colostrum

Antibody at birth		No. of calves tested	Type of antibody change		
ID	VN		1	2	3
—	—	25	0	0	25
—	+	3	0	2	1
+	+	8	4	1	3

ID: Immunodiffusion; VN: Virus neutralizing.

5 and 6 months of age, respectively. This decreasing prevalence of seropositive calves roughly coincided with the results reported by other authors [3, 21]. In this study, in which 76 calves were tested, the longest persistence of colostral antibody was found to be about 5 months. This persistence period was similar to that reported by Fishcer and Keyserlingk-Eberius [9] who studied 21 calves, but shorter than that (about 6 months) reported by BurrIDGE *et al.* [3] and Thurmond *et al.* [21] who used 139 and 130 calves, respectively. Therefore, it is suggested that if more calves had been investigated in this study a few of them would have had colostral antibody detectable by the ID test, even at 6 months of age.

Changes in antibody titers of infected calves could be divided into three types (Fig. 5). Type 1 changes could be differentiated readily at an early postnatal age from changes observed in uninfected calves. Type 2 changes could be differentiated at 5 to 7 months of age. Type 3 could not be differentiated from those of uninfected calves by the end of the observation period, 8 to 10 months, by the ID test. Although some calves with this type were found to be infected by the increase in VN antibody which occurred 2 to 3 months after disappearance of the pre-existing VN antibody, it was not clarified whether the increase was caused by reactivation of the primary infection or reinfection.

Results of our investigations and previous studies [3, 9, 21] suggest that the following tests are applicable to the diagnosis of BLV infection in early postnatal life: 1) tests for antibodies in precolostral serum by ID and VN, 2) comparison of antibody titers of two sera collected with an interval of about 1 month. This test can detect infected

calves showing Type 1 antibody changes, 3) tests for antibody titers at 5 and 6 months of age. Antibody titers higher than 2 will be regarded as virus induced antibodies, 4) tests at 7 months of age or older. All positive reactors will be regarded as infected animals.

To prevent contact infection by BLV among a group of calves born to seropositive dams it is necessary to detect all infected calves by 7 months of age since most of the uninfected calves changed to VN-antibody negative at 7 months of age (Fig. 3). Previous results showed that infection occurs in calves older than 7 to 10 months of age [18].

Changes in antibody titers of calves infected in utero with BLV were divided into three types. These types seemed to be caused by differences in the degree of virus propagation in vivo and/or immunological disorders in the host animals due to in utero virus infection. An increase in the number of virus-bearing cells is thought to stimulate an increase in serum antibody titers [10]. On the other hand, immunological modification such as tolerance is sometimes caused by in utero virus infection [12, 16]. It remains to be explored whether calves showing Type 3 antibody response carry virus-producing lymphocytes and play a role as an infectious source of BLV.

The in utero BLV transmission rate has been reported to be 3% [8], 0 and 13% [23] and 18 to 20% [7, 18]. The results obtained in the present study, using precolostral sera, showed a prevalence as high as 26%. The main reason for this high rate is the very high positive rate (47.1%) observed in a one herd (Table 1). The average positive rate in the remaining three herds was 15.2%. The cause of this unexpectedly high positive rate in the one herd is unknown. The fact that most of the calves that had

precolostral antibody in the herd showed antibody changes of Type 1 or 2 indicates in utero BLV infection and denies the possibility of error in observation of ingestion of colostrum. Of the calves that were not tested for precolostral antibody, 11.7% of the animals that showed antibody change of Type 1 or 2 were diagnosed as having been infected with BLV in utero. This diagnosis was supported by successful virus isolations performed on the calves. This result showed that the test for sequential antibody titers for BLV is a useful method for detecting BLV-infected animals in the early postnatal period, although this positive rate was lower than the average positive rate observed by using precolostral sera. This low rate might be partly due to failure to detect animals showing antibody changes of Type 3. Some animals showing Type 2 antibody change also might not be detected by the ID test since the observation period was not long enough.

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要 約

牛白血病ウイルス子宮内感染子牛の血清学的検出法： 甲野雄次・泉対 博・新井啓五・藤垣 彰¹⁾・榎本千可志¹⁾・岩崎充祐²⁾・石田秀史³⁾（家畜衛生試験場，¹⁾大分県大分家畜保健衛生所，²⁾宮崎県宮崎家畜保健衛生所，³⁾新潟県中央家畜保健衛生所）——牛白血病ウイルス（BLV）の子宮内感染率を知る方法および子宮内感染子牛を生後早期に検出する方法を検討した。4牧場のBLV抗体陽性母牛由来の初乳摂取前子牛50頭のうち10頭（20%）は寒天ゲル内沈降（ID）反応で，13頭（26%）は中和反応で抗体陽性であり，子宮内で感染したと考えられ，とくに1牧場では陽性率47.1%であった。BLV抗体陽性牛由来の子牛では，初乳摂取後全例がID抗体陽性となり，移行抗体はBLV非感染牛では約20日の半減期で減衰し，6か月後には全例陰性となった。一方，子宮内感染子牛16頭では，6か月以降でも多数がID抗体陽性を示した。これらの個体のID抗体価を経時的に調査すると，1）一定レベルの抗体価がおおむね持続する（6例，37.5%），2）一時的に非感染牛と同じく減少した後再び上昇する（6例，37.5%），3）同じく一時減少した後完全に消失する（4例，25.0%），3型にわけられた。初乳摂取前血清を検査できなかった子牛60頭については，7頭（11.7%）が1）および2）の型に属し，うち5頭ではBLV感染が確認された。