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Vector transmission of bovine leukemia virus during summer season in Northern Hokkaido

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Abstract

One of the known transmission pathways of bovine leukemia virus (BLV) is bloodsucking insects. Against this background, this study investigated changes in BLV seroconversion in cattle by season on three private farms in Northern Hokkaido. Study results showed that no BLV seroconversion was observed during winter, a season without horse flies, and that all seroconversions occurred during summer. Thus, we collected horse flies which were observed sucking blood on cattle in public grazed fields and performed BLV detection tests in the insects. The tests showed that 75% of the total collected horse flies were BLV-positive. These results suggested the existence of vectors such as horse flies in grazing filed (in summer) was a risk factor of the spread of BLV infection in Northern Hokkaido.

Key Words: Bovine leukemia virus, horse fly, vector transmission

Bovine leukosis is classified into two types: endemic enzootic bovine leukosis (EBL) caused by bovine leukemia virus (BLV); and non-infectious sporadic bovine leukosis (SBL) of unknown cause, which has no association with BLV. Both types are pathologically characterized by associated neoplastic lymphocytosis and systemic lymphoma, which may cause the affected animals to die. While the majority of BLV-infected cattle are aleukemic, 20-30% of the total BLV-infected cattle develop persistent lymphocytosis, in which the abnormal hyperplasia of polyclonal B cells presents. Further, 2-3% of infected cattle will develop EBL, which is clonal or oligoclonal B cell leukemia. In cattle with EBL, malignant lymphosarcoma is formed in the lymph nodes, the outcome of which is poor, causing the animals to die[10)]. The onset of EBL occurs mainly in adult cattle of 3 years of age or older. Not all BLV-infected cattle present the onset of lymphoma. However, if a cow is infected with BLV, its milk production level declines even though the animal presents no symptom[7,11)]. Thus, BLV infection in cattle causes significant economic loss.

In the light of the fact that in Hokkaido, there has recently been a tendency for an increase in

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Table 1. History of seroconversion of BLV-infection in the tested farms in Northern Hokkaido.

	Initial test †	%. of seroconversion †,‡					
	%. of seropositive cattle	2010	2011		2012		2013
		Nov.	Apr.	Nov.	May	Nov.	Jul
Farm A	79.5% (31/39)	36.4% (4/11*)	0% (0/9*)	50.0% (6/12*)	0% (0/17*)	19.0% (4/21*)	N.D
Farm B	63.0% (29/46)	57.9% (11/19*)	0% (0/9*)	77.8% (7/9)	0% (0/6*)	50.0% (4/8*)	N.D
Farm C	63.3% (19/30)	N.D #	N.D	33.3% (4/12*)	0% (0/13*)	69.2% (9/13)	0% (0/4)

* Some seronegative cattle were introduced from other herds in the own farms.
† BLV infection was tested by the passive hemagglutination assay (Nippon Institute for Biological Science, Ome, Japan).
‡ Seroconversion: % = (No. of positive cattle/No. of negative cattle at last test) x100.
# N.D: not demonstrated.

the local incidence rate of EBL caused by BLV (http://www.maff.go.jp/j/syouan/douei/kansi_densen/kansi_densen.html), urgent disease control is required. In recent years, it has been pointed out that the dehorning, cattle barns for free range farming and a large number of horse flies during summer are associated with an increase in seropositive rate among cattle on dairy farms. Another study has reported that there is a tendency for an increase in positive conversion rate during the grazing period if the positive rate among the cattle group is high at the beginning of grazing and if the number of bloodsucking insects on the farm is large[3,4]. In Northern Hokkaido, grazing farming in summer which takes advantage of its cool climate is widely adopted. On the other hand, since a large number of various kinds of bloodsucking flies have been observed on grazing fields in the area, it can be considered that summertime grazing and the existence of bloodsucking flies may be risk factors of BLV transmission on the farms. However, no detailed report has been published on the risk of BLV transmission via flies in the Northern Hokkaido. Thus, this study regularly performed BLV serological tests over the course of 3 years on 3 dairy farms in Northern Hokkaido, in which grazing had been adopted as the primary method of breeding. The time and factors of BLV infection were estimated based on changes in seropositive conversion rate. Additionally, we collected horse flies which were observed sucking blood on cattle on public grazed fields in summer in Northern Hokkaido and performed BLV detection tests in the insects.

The tested three farms with stall barn had been conducted BLV-control measures to prevent vertical transmission via colostrum and iatrogenic infections via contaminated materials. In the farms, cows (Holstein lactating cows, aged 6 months and older) were grazing during the daylight hours from May to October. In this study, BLV infection was tested by the passive hemagglutination assay (Nippon Institute for Biological Science, Ome, Japan). The initial test was performed in cattle on Farms A and B prior to grazing in April 2010. Among all tested cows, BLV seropositive was observed in 79.5% and 63.0% on Farms A and B, respectively (Table 1). In tests performed following the end of grazing in November of the same year, among BLV negative-cattle on Farms A and B, seroconversion was observed in 36.4% and 57.9%, respectively. However, no seroconversion was observed during winter until April of the following year. In tests performed following the end of grazing in November in 2011, seroconversion was again observed at high rates (50.0% on Farm A, 77.8% on Farm B); however, no cattle presenting seroconversion were observed during winter. The same tendency was observed in 2012, and it was confirmed that seroconversion occurred only during summer. Also, on Farm C, while the seroconversion rate was high in summer, no cattle

Table 2. Detection of BLV in horse flies captured from the infected cattle at public grazed fields in Northern Hokkaido

Cattle		Tabanid flies*						
		No. of				BLV detection †		BLV sequence ‡
Cattle ID	BLV-infection †	tested flies	BLV-positive flies †	Fly ID	Species	Sucking mouth-part	Midgut	Cattle vs Fly
1	+	2	2 (100%)	1-1	*T. chrysurus*	+	N.D	N.D
				1-2	*T. chrysurus*	+	N.D	N.D
2	+	6	3 (50%)	2-1	*T. chrysurus*	+	+	Mismatch (1bp)
				2-2	*T. chrysurus*	+	N.D	N.D
				2-3	*T. chrysurus*	+	-	N.D
				2-4	*T. chrysurus*	-	N.D	
				2-5	*T. chrysurus*	-	N.D	
				2-6	*T. nipponicus*	-	N.D	
3	+	4	2 (50%)	3-1	*T. chrysurus*	-	+	N.D
				3-2	*T. chrysurus*	-	+	Complete match
				3-3	*T. nipponicus*	-	-	
				3-4	*T. nipponicus*	-	-	
4	+	1	1(100%)	4-1	*T. chrysurus*	+	+	Complete match
5	+	2	1(50%)	5-1	*T. chrysurus*	+	-	N.D
				5-2	*T. chrysurus*	-	N.D	
6	+	1	1(100%)	6-1	*T. chrysurus*	-	+	N.D
7	+	1	1(100%)	7-1	*T. chrysurus*	-	+	N.D
11	+	1	1(100%)	11-1	*T. chrysurus*	+	+	Mismatch (1bp)
13	+	1	1(100%)	13-1	*T. nipponicus*	-	+	Complete match
14	+	2	2(100%)	14-1	*T. chrysurus*	+	+	N.D
				14-2	*T. chrysurus*	+	+	N.D
15	+	5	5(100%)	15-1	*T. chrysurus*	+	+	Complete match
				15-2	*T. chrysurus*	-	+	N.D
				15-3	*T. chrysurus*	+	N.D	Mismatch (1bp)
				15-4	*T. chrysurus*	-	+	N.D
				15-5	*T. chrysurus*	+	-	N.D
17	+	2	1 (50%)	17-1	*T. chrysurus*	-	-	
				17-2	*T. chrysurus*	-	+	Complete match
Total		28	21(75%)			13/28 (46.4%)	14/20(70%)	

* Attaching horse flies on cattle were captured using net. Captured flies were examined microscopically to determine their species identities.

† BLV infection was tested by the nested PCR as previously described (Fechner et al., 1997). +: positive, -: negative, N.D: Not demonstrated.

‡ The BLV env gene from the infected cattle and flies was identified and compered by sequence analysis as previously described (Ooshiro et al., 2013). N.D: Not demonstrated.

presented seroconversion during winter (Table 1). Since seroconversion was only observed during summertime grazing and bloodsucking flies were not observed in the farms in winter, we collected horse flies, which were observed sucking blood on cattle on public grazed fields in summer in the area and performed BLV detection tests in the insects. Using a net, we collected 28 horse flies from 17 cattle. The species of collected horse flies were identified under a microscope. The horse flies were identified as *Tabanus chrysurus* or *Tabanus nipponicus*. Proviral DNAs were extracted from the horse fly's mouthpart and midgut using the commercial kit (SepaGene, SankoJunyaku Co., Ltd. Tokyo, Japan) and detected by nested PCR method targeting the *envelope (env)* gene region[2]. To detect BLV in certain horse flies, their DNAs were extracted with 100 μg of their midgut contents emulsified with PBS. Additionally, the DNAs of cattle from which the horse flies were collected were extracted from their blood to perform BLV detection tests. BLV-positive PCR products obtained from the horse flies and cattle were analyzed to identify their env gene sequences through a sequencing method as previously described[9]. The results of analysis detected BLV in the mouthpart or midgut of 21 horse flies (75%) out of 28 which were sucking the blood of BLV-infected cattle (Table 2). Among 8 horse flies in which the env gene region of BLV was identified, such a region in 3 was different by one base from that of *env* genes collected from the cattle (Table 2). However, the gene sequences of such regions in the 3 horse flies were the same as those of other BLV-infected cattle in the same barn (data not shown).

This study continuously performed serological tests in groups of cattle on three dairy farms in Northern Hokkaido before and after grazing over the course of 3 years. While no cows presented seroconversion during the winter period in which they were kept in barns, high seroconversion rates were observed through tests performed after the end of grazing. No horse flies were observed in the barns in the winter seasons. These findings suggested that BLV infection is mainly occurring during the summertime grazing period. It was suspected that bloodsucking insects, such as horse flies, were associated with BLV transmission. In fact, BLV were detected from the horse flies collected in grazing fields. Also, the env gene sequence of such genes was the same as that of the genes from BLV-infected cattle in grazing fields. These findings suggested that horse flies are associated with BLV transmission during the grazing period.

BLV is transmitted via bloodsucking insects such as horse flies[8] and stable flies[1]. In recent years, cases, in which BLV seroconversion was successfully suppressed through vector control in Japan, have been reported[6,9]. As in this study, other reports have stated that BLV seroconversion declines in winter in Hokkaido; therefore, it is considered that bloodsucking insect control in summer is important. As an example of bloodsucking insect control, a study has reported that using fly nets can effectively prevent BLV infection[6]. However, it is extremely difficult to completely eliminate bloodsucking insects on the farm. According to a report by Buxton *et. al.,* stable flies (mouthpart) which have sucked blood from BLV-infected cattle show no infectivity within 1 hour of sucking the blood. Therefore, it can be considered that to prevent BLV infection, it is important to prevent such insects from sucking blood from cattle at short intervals. It has been reported that the risk of BLV infection increases when BLV-uninfected cattle are kept next to BLV-infected cattle in tie-stall facilities. Therefore, it has been considered that keeping infected cattle and negative ones separate from each other may be an effective in tie-stall facilities[5]. A large number of trials have verified that separating BLV-infected cattle from uninfected ones during grazing in the field and in free-stall barns, is effective in preventing BLV transmission via bloodsucking flies such as horse flies. This study looked into the situation of BLV seroconversion among cattle in Northern Hokkaido, the coldest area in Hokkaido. The study suggested that, as in other areas, BLV infection is transmitted via bloodsucking insects such as horse flies during the short summer season in Northern Hokkaido. It was found that it is important to take BLV control measures during summertime going forward.

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