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NOTE

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Vector control efficacy of fly nets on preventing bovine leukemia virus transmission

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ABSTRACT. Bovine leukemia virus (BLV) is horizontally transmitted among cattle through infected blood. This 3-year field study (2013–2016) aimed to confirm the potential of the blood-sucking stable fly as a risk factor of BLV transmission and to determine the efficacy of vector control on preventing the transmission of BLV. The BLV-positive conversion rate during summer was higher than that during winter in a model dairy farm, where many stable flies were observed during the summer. After fly nets were fixed onto the barn to prevent fly invasion, the BLV-positive conversion rate during the summer was significantly decreased compared with that in the absence of fly nets (P<0.01). These findings suggest that vector control using a fly net may inhibit BLV transmission.

KEY WORDS: bovine leukemia virus, fly net, risk factor, stable fly, vector control

Bovine leukemia virus (BLV) is an oncogenic retrovirus and a causative agent of enzootic bovine leukosis (EBL). Once cattle are infected with BLV, they remain infected for life. However, the majority of infected cattle do not display clinical signs of the disease. Persistent lymphocytosis (PL) will occur in approximately 30% of infected cattle, and fewer than 5% of them will develop malignant lymphoma [3, 10]. In Japan, the incidence of EBL has been increasing since 1997, and the spread of BLV infections is a great concern [16].

In general, BLV is horizontally transmitted among cattle through infected lymphocytes in the blood. Iatrogenic transmission was considered to be responsible for most new infections, including common use of rectal palpation sleeves, injection needles, and dehorners [7, 12, 13]. BLV can also be transmitted by blood-sucking insects such as tabanid and stable flies. Mechanical transmission of BLV by virus-infected lymphocytes on the mouthparts of flies has been experimentally demonstrated [4, 6, 17, 20]. Although BLV transmission might occur in regions with high populations of blood-sucking insects, the role of the insects in BLV transmission under natural conditions is still uncertain. Field studies have reported that increased prevalence of BLV infection in the summer season was associated with the activity of the horsefly population, while other prospective studies observed no seasonal variation in the BLV seroconversion rate [14, 15, 23]. The proviral load in BLV-infected cattle is an important factor of BLV transmission via insect vectors, and PL cattle, which have a high proviral load, become infectious sources in farms [9, 19]. Although previous studies considered that blood-sucking insects were low risk factors for BLV transmission [8, 18, 24], transmission might be dependent on the density of insects around the cattle, the proportion of BLV-infected cattle within farms, and the level of proviral load in BLV-infected cattle [1].

The objectives of our study were to investigate the potential of stable flies as a risk factor for BLV transmission under natural conditions in dairy farms and to determine the efficacy of a fly net in controlling vector insects of BLV.

This field study was conducted at a model farm (Dairy farm A) in eastern Hokkaido, Japan. One of the dairy cows in the farm developed EBL in 2003, and the BLV prevalence rate in cows was 52.1% at the beginning of this study in July 2013 (Table 1). Dairy farm A housed 270 Holstein cows including 150 dairy cows. These dairy cows were housed in freestall barns and were not allowed to graze on pasture. During the dry period, the cows were kept in a dry cow barn for about 6–8 weeks and they returned to the freestall barn of milking cows with milking parlor after calving. To prevent BLV transmission within the farm, pasteurized colostrum feeding, single use of needles and plastic sleeves, and disinfection of dehorning and hoof cutting instruments were implemented.

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Table 1. Bovine leukemia virus (BLV) prevalence rates and numbers of lymphocytic cows from farm A

Year	2013	2014	2015	2016
Month	July	March	April	April
Number of cows tested	263	261	284	255
BLV prevalence rate (%)	52.1	49.4	36.2***	29.0****
Number of lymphocytic cows[a)]	19	22	18	13
Proviral load in lymphocytic cows (copies/μl)	18,946 ± 10,670	18,090 ± 9,999	17,961 ± 8,461	13,346 ± 3,073
Ratio of lymphocytic cows (%)	7.2	8.4	6.3	5.1

a) Lymphocytic cows were detected according to absolute lymphocyte count and age based on European Community's key. ***P<0.001, ****P<0.0001, compared with 2013.

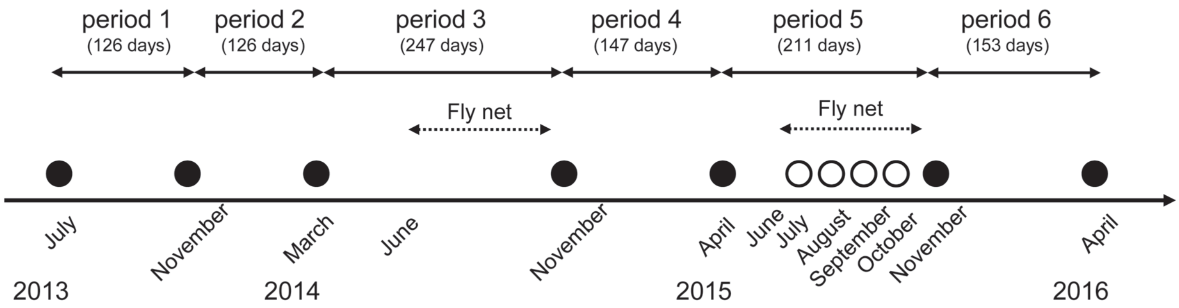

Fig. 1. Blood sampling and fly trapping protocols. Blood of dairy cows (●) was collected seven times from July 2013 to April 2016. Fly nets were fixed onto the wall of a freestall barn of milking cows from June to November in 2014 and 2015. Flies (○) were trapped in insect bags in both milking cow and dry cow freestall barns once a month from July to October 2015.

However, planned elimination or segregation of BLV-infected cattle was difficult, and thus vector insects were not controlled.

According to interviews with farmers in 2013, stable flies were frequently observed in both the barns for milking cows and dry cows from August to October. To prevent the invasion of stable flies into barns and decrease the occurrence of BLV transmission by blood-sucking flies, a commercially available fly net (SC Environmental Science Co., Ltd., Osaka, Japan) was fixed onto the wall surrounding the freestall barn of milking cows from June to November in 2014 and 2015. The fly nets measuring 2 m in width, with a 6 × 6 mm mesh size, contained pyrethroid insecticides. The dry cow barn remained unprotected against flies without the fly net.

The overall study period spanned 3 years from July 2013 to April 2016, and was divided into six periods: periods 1, 3, and 5 included the summer season, while periods 2, 4, and 6 included the winter season (Fig. 1). Blood samples from dairy cows were collected seven times at the beginning and end of each period. The BLV prevalence rate in cows was confirmed by detecting anti-BLV antibodies using a commercial ELISA kit (JNC Inc., Tokyo, Japan) at the beginning of periods 1, 3, and 5 and the end of period 6. Lymphocytic cows were detected according to absolute lymphocyte count and age based on European Community's Key [2]. Genomic DNA was isolated from the blood of lymphocytic cows using the Wizard Genomic DNA Purification Kit (Promega, Tokyo, Japan), and proviral load was quantified using BLV-CoCoMo-qPCR (RIKEN Genesis, Tokyo, Japan). New cases of BLV infection were screened by detecting anti-BLV antibodies and confirmed by detecting BLV provirus using nested PCR targeting the BLV long terminal repeat region [22]. The BLV-positive conversion rate in dairy cows was calculated as the number of new cases of BLV infection during each survey period. The number of flies in the barns was counted to demonstrate the effect of fly nets on the prevention of flies entering barns. Flies were trapped in insect bags between 2 p.m. and 3 p.m. in the milking cow and dry cow barns, once a month from July to October 2015. Stable flies were morphologically identified under a stereomicroscope, and DNA was extracted for PCR analyses targeting the bovine leukocyte antigen DRA gene [21] to confirm that they sucked cow blood. The presence of BLV provirus in the fly was also analyzed.

Fisher's exact test was used to compare the BLV prevalence rate of cows in later periods to that of July 2013 and the BLV-positive conversion rate between each survey period. The unpaired t-test was used to compare the number of stable flies in cow barns with or without fly nets. Differences were considered significant at P<0.05.

The BLV prevalence rate of cows in March 2014 was 49.4%, which was similar to that in July 2013. After employing the fly net on the barn of milking cows from June to November 2014, the BLV prevalence rate in April 2015 significantly decreased to 36.2% (P<0.001) compared with the rate in July 2013 (Table 1). The prevalence rate in April 2016 was slightly decreased compared with that in April 2015. Foot disease, gastroenteric disorder, and abomasum displacement occurred frequently in 2014 and 2015. Affected cows, which included BLV-infected cows, were removed from the farm, which might have contributed to the decreased BLV prevalence rate.

Nineteen lymphocytic cows were detected in July 2013, and their mean (± standard deviation) blood proviral load was 18,946 ± 10,670 copies/μl (Table 1). Most of the BLV-infected cows with PL were not eliminated during the study period, and the ratio of

Table 2. Bovine leukemia virus (BLV)-positive conversion rates in dairy cows from July 2013 to April 2016

Survey period[a)]	1	2	3	4	5	6
Year	2013	2013–2014	2014	2014–2015	2015	2015–2016
Days in the period	126	126	247	147	211	153
Season	Summer/Fall	Fall/Winter	Spring/Summer/Fall	Winter/Spring	Spring/Summer/Fall	Winter/Spring
Number of cows tested[b)]	74	60	82	90	98	99
Number of BLV non-infected cows	59	59	75	88	93	96
Number of BLV infected cows[c)]	15	1	7	2	5	3
BLV-positive conversion rate (%)	20.3	1.7**	8.5*	2.2**	5.1**	3.0**

a) Period 1: July–November 2013; period 2: November 2013–March 2014; period 3: March–November 2014; period 4: November 2014–April 2015; period 5: April 2015–November 2015; period 6: November 2015–April 2016. b) BLV non-infected cows at the beginning of the survey period. c) The number of cows newly infected with BLV during the survey period. *$P<0.05$, **$P<0.01$, compared with period 1.

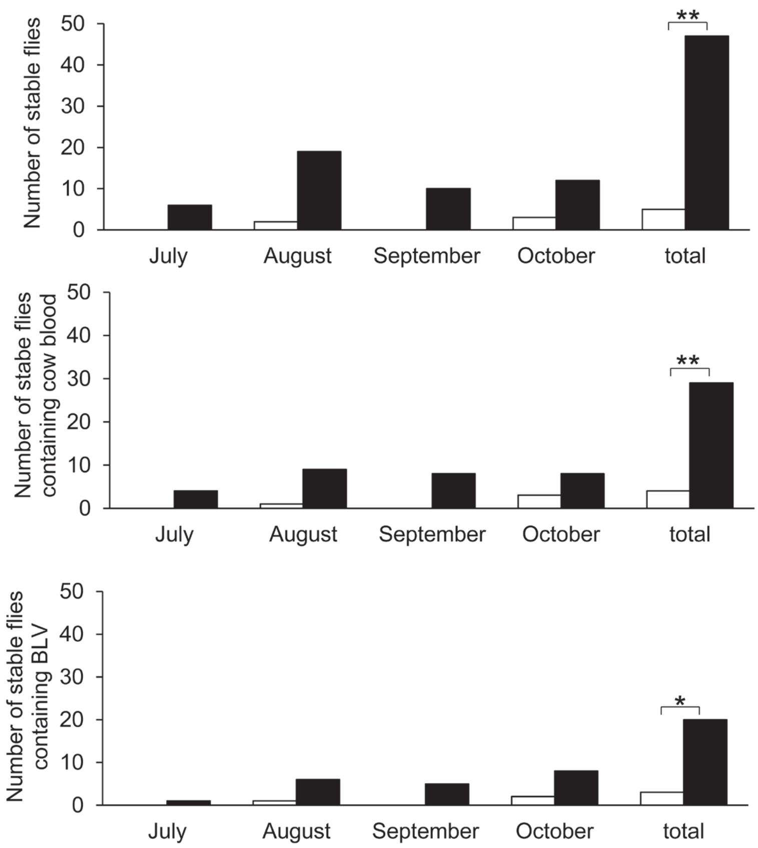

Fig. 2. Number of stable flies in a milking cow barn with fly nets (□) and in a dry cow barn without fly nets (■). Stable flies were trapped in insect bags once a month from July to October 2015 (*$P<0.05$, **$P<0.01$).

lymphocytic cows remained about the same from July 2013 to April 2016.

In survey period 1, from July to November 2013, the BLV-positive conversion rate in dairy cows was 20.3%, which was significantly higher than that in period 2 (1.7%) from November 2013 to March 2014 ($P<0.01$) (Table 2). In survey periods 3 and 5, which included the summer and fall seasons, milking cows housed in the freestall barn with fly nets had significantly decreased BLV-positive conversion rates of 8.5 and 5.1% ($P<0.05$ and $P<0.01$), respectively, compared with the rate in period 1 without fly nets on the stall (Table 2).

The survey of stable flies during the summer and fall of 2015 revealed that the stable flies containing cow blood and BLV provirus were present in the dairy cow barns. This finding suggested that the blood-sucking stable flies might be responsible for transmission of BLV within dairy cows. The numbers of all flies and ones containing cow blood and BLV provirus were significantly lower in the barn with fly nets than in the barn without fly nets ($P<0.01$, $P<0.01$, and $P<0.05$, respectively) (Fig. 2). It may be difficult to directly compare the number of flies between the milking cow barn and the dry cow barn because of the location, building size, and number of cows that differed between these buildings. However, this finding suggested that the fly net decreased the number of flies in the milking cow barn. Follow-up interviews with farmers in November 2014 revealed that stable flies were rarely seen in the freestall barn of milking cows after employing the fly net, confirming that the fly net had a beneficial effect on the prevention of fly invasion.

Seasonal variations in the BLV-positive conversion rates were observed, and the rate in the summer was higher than that in the winter (Table 2). The presence of many stable flies from July to October suggested that blood-sucking flies might be responsible for BLV transmission in the summer season, in accordance with previous studies [15, 18]. However, the BLV-positive conversion rate was very low in the winter season. These observations suggested that the presence of blood-sucking insects should be considered as a greater risk factor of BLV transmission than direct contact between infected cows and healthy cows. Some statistical analyses presented blood-sucking insects and a lack of controlling flies as risk factors [5, 11]. Additionally, a field study noted that controlling vector insects with insect repellents efficiently prevented the spread of BLV infection [19]. In our study, with the fly net fixed onto the barn to prevent fly invasion, the BLV-positive conversion rate during the summer decreased significantly compared with that under the condition without fly nets. The decreased BLV-positive conversion rate could lead to a decrease in the BLV prevalence rate of cows. These findings indicated that controlling vectors with fly nets in dairy farms is very useful for decreasing new BLV infections without culling PL cows or segregating infected cattle.

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