FULL PAPER *Virology*

Sero-epidemiological analysis of vertical transmission relative risk of Borna disease virus infection in dairy herds

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ABSTRACT. Borna disease virus (BDV) is a virus that causes a neurological disease in domestic animals, including a variety of animal species in Japan. Few studies have examined the mode of transmission of this virus in cattle, and the exact mechanisms underlying the transmission of the virus need to be elucidated. This study aimed to examine the contribution of vertical transmission of the virus, which occurs when the virus is transmitted from the mother to offspring during gestation or birth. We used an epidemiological approach. The relative risk (RR) was calculated for cattle born to BDV sero-positive cows from farms with a higher within-herd prevalence of BDV (56.8%). We tested the sera of 1,122 dairy cattle from 24 dairy herds in Hokkaido Prefecture, Japan, for BDV infection using the ELISA and western blotting method. The overall level of BDV sero-prevalence was 22.1%. Seroprevalence was significantly higher in closed-breeding herds that do not have buying in cows (39.7%) than in farms that restock cattle by buying in cows (4.4%, $P<0.01$). The overall RR of BDV vertical transmission from infected mothers to their daughters was 1.86 (95% confidence interval (CI): 1.54–2.56). Our results show that vertical transmission contributes significantly to BDV transmission in the farms tested in this study.
KEY WORDS: Borna disease virus, dairy cow, relative risk, vertical transmission

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Borna disease virus (BDV) is a virus that causes a neurological disease, which is named after an epidemic of the disease in horses in 1885 in the town of Borna in southern Germany. Borna Disease (BD) by infection with BDV causes a neurological disease with non-suppurative encephalitis [10, 11, 16, 22]. BD occurs in many animal species worldwide, including Japan. However, cases of BD with neurological symptoms in cattle are rare. Symptoms other than encephalitis, such as infertility or astasia, has been confirmed in antibody-positive cows [1]. Borna disease in both domestic animals and companion animals has been reported in Japan [3–5, 7, 8, 13–15, 25]; however, epidemiological studies of cattle are lacking. There are few studies on the mode of transmission of this virus in cattle, and many questions remain surrounding infection. The method by which antibody-positive cattle were infected remains unknown. Most dairy farmers in Hokkaido Prefecture, Japan, have introduced young cows from other farmers to expand the scale of their farms following the herd size increase in recent years [18]. Many farms in Hokkaido have traded large numbers of cattle in recent years. It has been recognized as a general dairy management. Conversely, some dairy farmers manage their business using a closed breeding herd model. In other words, the herd has the same maternal origin. In this study, we aimed to quantify the seroprevalence of BDV within farms and the virus transmission rate between cows within the same family. Therefore, we investigated the association between calves born from BDV-seropositive mothers, and seropositivity in daughters to understand the contribution of vertical transmission to total BDV transmission.

MATERIALS AND METHODS

Samples: buy-in heifers or closed-breeding. Farms were selected from those routinely visited for clinical purposes who agreed to participate in this study. A total of 1,122 Holstein dairy cows were serologically tested for BDV antibodies. Of those, 498 of the cows were from the 21 farms that would buy in cows from outside farms, while 624 of the cows were from three farms that would breed within closed herds, without buying in from outside farms (Table 1).

Detection of antibody: Serum samples were diluted to a ratio of 1: 100 with phosphate buffered saline containing 10% Block Ace (Dainippon Pharmaceutical Co., Osaka, Japan) and 0.05% Tween 20. The samples were tested for BDV antibodies using the enzyme-linked immunosorbent assay (ELISA) to screen for the recombinant BDV nucleoprotein (BDV-N) antigen as described in our previous studies [2]. To detect antigen-bound bovine immunoglobulin, we used a peroxidase-conjugated goat affinity purified anti-bovine IgG (Bethyl Laboratories, Inc., Montgomery, TX, U.S.A.). Positive reactions were identified using a Microplate Imaging System (Ultramark, Bio-Rad, Hercules, CA, U.S.A.) at 405 nm. The cutoff value for ELISA was calculated as the

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Table 1. Cows group Overview and BDV seroprevalence in the breeding herd

	Non-closed breeding herd		Closed breeding herd					
	Heifer	Adult	Heifer			Adult		
			A	B	C	A	B	C
BDV(+)	13	9	4	24	15	70	74	61
BDV(−)	366	110	16	70	9	123	123	35
Positive rate	3.4 (13/379)	7.6 (9/119)	20 (4/20)	25.5 (24/94)	62.5 (15/24)	36.3 (70/193)	37.6 (74/193)	63.5 (61/96)
	4.4 (22/498)		31.2 (43/138)			42.2 (205/486)		
			39.7 (248/624)					
	24.1 (270/1122)							

mean ± 2SD at an optical density (OD) of 405 nm from 5 intact cows (cutoff: OD, 0.4). ELISA-positive samples were further examined to confirm their specificity to the BDV-N antigen. Antibody-antigen complexes were detected using the same peroxidase-conjugated goat affinity purified anti-bovine IgG [9].

To analyze the contribution of vertical transmission to overall virus transmission, we selected closed breeding herds with no history of buying in for at least 20 years. Using the statistical software R (version 3.0.2), we calculated the relative risk (RR) for seropositivity in daughters born to mothers who were BDV-seropositive. The relative risk is an indicator to evaluate the strength of association between the exposure to a factor and disease in a cohort study. It is calculated as a ratio of the incidence rate of exposed group to that of non-exposed group by a certain factor.

RESULTS

In our study, the overall sero-prevalence of BDV was 24.1% (270/1122), and there were entire farms with BDV sero-positive cows [Table 1]. There were no BD cases reported during the study period, and all the cows were in a healthy condition. All three closed-breeding farms had BDV sero-positive animals, with a prevalence of 39.7% (248/624). The prevalence in the other 21 non-closed breeding (buy-in) farms was 4.4% (22/498), which was significantly lower than that of the closed-breeding farms (x^2=187.2, df=1, P<0.01).

To investigate why prevalence differed between the closed-breeding and buy-in farms, we examined the stage of the animals that were sero-positive. In closed-breeding farms, sero-positivity in adult cows was 42.2% (205/486), which was significantly higher than in heifers (31.2%, 43/138; x^2=5.0, df=1, P=0.03). This suggests an increase in prevalence as the cows age, due to increasing exposure to the virus over time. Conversely, in buy-in breeding farms, prevalence was not significantly different between heifers (3.4%, 13/379) and adult cows (7.6%, 9/119; x^2=2.8, df=1, P=0.1, Table 1). In the closed-breeding farms, the BDV sero-prevalence of adult cows from individual farms were: farm A 36.3% (70/193); farm B 37.6% (74/197); and farm C 63.5% (61/96) (Table 1). The prevalence in these farms was approximately 6 times higher than that in non-closed breeding farms as shown in Table 1. The proportion of adult cows that were sero-positive was significantly greater in closed-breeding farms (77.8%, 486/624) than in non-closed breeding farms (23.9%, 119/498; x^2=322.8, df=1, P<0.001). The greater proportion of sero-positive adult cows in the closed-breeding farms was the major contributor to the different levels of sero-prevalence detected between closed and non-closed breeding farms.

Table 2. Cohort study on vertical transmission in the closed breeding herd

		Adult		
		BDV (+)	BDV (−)	Total
Heifer	BDV (+)	54	50	104
	BDV (−)	41	106	147
	Total	95	156	251

Table 3. Relative risk of BDV vertical transmission in the closed breeding herd

	A	B	C	Entirety
Vertical transmission relative risk	1.01	1.94[a)]	1.67	1.86[a)]
95% confidence interval (Lower ~ Upper limit)	0.56~1.82	1.27~2.98	0.99~2.82	1.54~2.56

a) Significant at the 95% confidence interval.

We investigated the RR of seropositivity of daughters using sero-epidemiological data. The combination was able to confirm the information of the mother and child relationship was 251 pairs (Table 2). Of the 251 sets, the relationship of mother and daughter has been established; the mother was positive was 95 pairs (95/251 37.8%), and out of the 95 sets, the daughter cows were positive was 54 pairs (54/95 56.8%). The RR was 1.01 at farm A, 1.94 at farm B, and 1.67 at farm C. Across all three closed breeding farms, the RR was 1.86 (95% confidence interval, (CI): 1.54−2.56) (Table 3).

DISCUSSION

Previous epidemiological studies on BDV infection in cattle have suggested that BD virus is transmitted within the herd. However, to date, the RR of BDV transmission

remains unclear. This study showed the seropositive rate of heifers from non-closed breeding (general dairy management) farms was 3.4%. Closed breeding herds did have a higher seropositive rate than non-closed breeding herds. If horizontal transmission was the major route for infection with this virus, the seroprevalence of BDV should be the same among them. However, the seroprevalence was similar in the non-closed breeding herds; therefore, there must be another transmission route that increased the seroprevalence of BDV in the closed-breeding herd. The non-closed breeding herd had contact with herds from other farms through pasture grazing, and the seroprevalence of BDV differed between the two farm management styles. Therefore, we focused on the possibility of virus transmission among the BDV-antibody positive herds. We analyzed the RR of vertical transmission between seropositive mothers and calves using epidemiological risk analysis, which was based on statistical techniques. This risk analysis strategy is used mainly to indicate the relationship between intensity of exposure factors and disease outbreaks. In humans, it has been widely used to calculate the relative risk between blood levels of the hepatitis C virus on liver cancer risk [19], as well as between passive smoking and lung cancer risk [24]. In this study, we also analyzed the occurrence of a causative agent for BSE. In the BSE report, Wilesmith showed the significance of vertical transmission in relative risk, with antibody-positive mothers, the postulated source of the agent exposure [26]. In this experiment, we used the statistical software R to evaluate the RR of BD virus transmission between BDV seropositive mothers and seropositivity in daughters. We found that the RR of BDV vertical transmission from infected mothers to their daughters was between 1.01 and 1.94 at three different closed-breeding farms. The RR across the three farms was 1.86 (95% CI: 1.54 ~2.56). This shows that vertical transmission was a significant RR in closed-breeding herds.

There are three different routes for vertical transmission. The first route is trans-placental transmission and intrauterine infection *in utero*, the second is that virus transmission occurs via the vagina during delivery, and the third is that the colostrum contains the virus or virus-infected cells [12, 21, 23]. BDV infection in equine or rodent fetuses has also been reported [6, 17, 20]. In this study, we calculated the RR of BDV vertical transmission in dairy herds for the first time. The antibody-positive ratio of cows from closed-breeding herds was significantly greater than in cows from non-closed breeding farms. Our statistical analysis suggests a strong possibility of vertical transmission of BDV. Closed-breeding herd management is likely to increase the rate of BDV transmission from seropositive mothers to their daughters by vertical transmission. Since seropositive cows are at risk of transmitting the virus to their calves, this might explain the higher BDV infection rates in closed-breeding farms. In the future, the detection and distribution of the virus should be evaluated in individual seropositive cattle.

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