NOTE *Virology*

A Case Control Study of Bovine Viral Diarrhea Virus (BVDV) Persistent Infection (PI) in Betsukai, Hokkaido, Japan

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ABSTRACT. The Betsukai town office implemented bovine viral diarrhea virus (BVDV) preventive activities (i.e., vaccination and surveillance) in 2006. Using bulk tank milk screening followed by individual blood tests using a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method, persistent infection (PI) cattle were detected and eliminated from the population. Based on data for PI cattle detected between 2006 and 2007, we conducted a case control study to find risk factors associated with the presence of PI cattle. Significantly associated farm level factors for increasing risk of producing PI cattle include; 1) no recent purchase of cattle (between 2004 and 2007) and 2) no prevention of people/animals entering the premises. This study suggests that not only vertical transmission from dam to calf but also indirect contact with people and animals play an important role in transmitting BVDV infection and subsequent production of PI animals.

KEY WORDS: biosecurity, BVDV infection, case control study, Hokkaido/Japan.

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Bovine viral diarrhea virus (BVDV) infection can result in decreases in body weight and milk production, reproductive disorder and in the worst case, death [1, 4, 5, 7, 14]. The main source of infection is a persistently infected (PI) animal derived from pre-natal infection, and thus, it is essential to test and slaughter PI animals to prevent BVDV infection [1, 15, 16]. Acute infection also contributes to production loss due to secondary infection caused by temporary defect in immunological responses [9]. Therefore, BVDV infection is recognized as a disease of serious financial loss in many countries where various control strategies have been practiced [1, 8, 12, 18].

Although Japan has no national level BVDV control programs, which are common practices in Europe, BVDV control programs have been launched in some parts of Hokkaido, which accounts for half of the total number of dairy cattle raised nationally. The pioneer in this effort is the township of Yubetsu, as reported in our previous papers [10, 17]. In Betsukai, mass vaccination and bulk tank milk (BTM) screening followed by individual blood tests using a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method (i.e., surveillance) took place in 2006 in collaboration with three local organizations, the town office, an agricultural cooperative (JA) and a mutual animal health insurance association (NOSAI). Most dairy farms produce their own animals on their farms but occasionally purchase animals from outside the town. It is reported in an epidemiological study conducted in Japan that cow-to-cow transmission frequently occurs at public breeding centers and results in the birth of PI animals [10, 17].

Vaccination prevents acute infection but not pre-natal infection [3]. Whenever BVDV control has been an issue, the European Community has stated that systematic control is the way forward if sustainable results and long-term effects are desired [1]. Systematic control puts more value on biosecurity, not vaccination. However, not many epidemiological studies on BVDV infection have been conducted in Japan. Therefore, as the first step, we applied a case control study method in order to identify farm level risk factors associated with the presence of PI cattle in Betsukai, Hokkaido, Japan.

Study area: Betsukai town is located in the Eastern part of Hokkaido. The main sources of income for the people of Betsukai are dairy/livestock production and fisheries. The town is densely populated with 881 dairy farms and a dairy cattle population of 107,800 [2]. In 2006 and 2007, 28 and 13 PI cows were identified on 18 and 5 farms, respectively. At the time of our study in 2008, there were five JAs to which most of the dairy farmers belonged. BVDV infection had been reported sporadically before 2006, which is when township-wide BVDV control was implemented. A very small proportion (3%) of cows had been vaccinated against BVDV prior to the 2006 vaccination campaign. During this 2-year campaign, animals were vaccinated twice a year using a live vaccine first and then an inactivated one.

Detecting PI cattle: BTM screening, followed by individual blood tests using a RT-PCR method, which has a sensitivity of 100% [11], was applied to find PI animals.

Selection of case and control farms: The target population was all dairy cattle in Betsukai town. The study was conducted at the farm level. A case was defined as a dairy farm that had at least one PI animal detected in either 2006 or 2007. A total of 15 case farms were included in the study. Twenty-nine control farms were selected from among the farms in the vicinity of the respective case farms with similar management methods that had tested negative in the

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BTM tests in 2006 and 2007.

Collection of farm data: A questionnaire consisting of 11 sections with 80 questions was created based on previous BVDV epidemiological research done in Europe [1] and Yubetsu, another smaller dairy town in the Eastern part of Hokkaido [17]. The questionnaire covered potential risk factors such as the number of purchased cattle between 2004 and 2007, use of public breeding centers and footpaths and the presence of wild animals in pastures. The questionnaires were delivered to the study farms by local JA offices. During farm visits in June and September, 2008, interviewers (two university undergraduate students) filled in missing answers and observed the premises of the study farms. For further investigation of PI cattle, we used the national cattle identification system (cattle traceability) to check individual animal movement records [13].

Statistical analyses: The farm was the unit of analysis, and all measurements were made at the farm level. In the first stage, an univariate analysis was applied. The measure of association between possible risk factors and the presence of PI cattle was examined for each factor individually. Categorical factors were evaluated for statistical significance using a chi-square or Fisher's exact test for independence. Means for non-categorical variables were compared using the Student's *t* test. In the second stage, a multivariate analysis was employed. Those risk factors having significant association ($p<0.05$) with the presence of PI cattle in the univariate analysis were evaluated using a multiple logistic regression model. This model was fitted using the Statistical Package for Social Scientists (SPSS) software (SPSS Inc., Chicago, IL, U.S.A.). Odds ratios and their 95% confidence intervals were calculated.

The cases and control farms were found to be very similar in general cattle management style. According to the univariate analysis, the following five factors were associated with the presence of at least one PI animal on a farm: no recent purchase of cattle (between 2004 and 2007), no footpaths for visitors, crows and cats observed in stables and no prevention of people/animals from entering the premises (for example, farmers do not put screens over windows). Finally, two factors, no recent purchase of cattle and no prevention of people/animals from entering the premises, were found to be significant in the multivariate analysis (Table 1). Table 2 shows details of the 15 case farms, five of which purchase animals. Three (Farms No. 2, 8 and 9) actually purchased PI animals before 2003. Concerning the ten farms that did not purchase animals between 2004 and 2007, only one dam was PI at farm No. 1, and the other nine farms

Table 1. Significant farm level risk factors associated with the presence of PI cattle in Betsukai, Hokkaido, Japan, in 2006 and 2007 (multivariate analysis)

	Coefficient	Odds ratio	95% CI	P value
Intercept	0.92			
Purchase of cattle between 2004 and 2007	–1.788	0.167	0.036–0.770	0.022
No prevention of people/ animals entering the premises	2.856	17.395	1.752–172.753	0.015

Table 2. Details of the 15 case farms in Betsukai, Hokkaido, Japan, with PI detected in either 2006 or 2007

Year PI animals detected	Farm No	Farm purchase status between 2004 and 2007	Number of PI animals detected	Dam status	Where PIs came from
2006	1	No	2	PI	Home bred
	2	Yes	1	Unknown	Purchased
	3	No	1	Negative	Home bred
	4	No	3[a)]	Negative	Home bred
	5	No	2	Negative	Home bred
	6	No	1	Negative	Home bred
	7	Yes	3[b)]	PI	Home bred
	8	Yes	1	Unknown	Purchased
	9	Yes	1	Unknown	Purchased
	10	Yes	1	Negative	Home bred
2007	11	No	1	Negative	Home bred
	12	No	4[a)]	Negative	Home bred
	13	No	1	Negative	Home bred
	14	No	5[c)]	Unknown/Negative	Purchased/Home bred
	15	No	2	Negative	Home bred

a) All their dams were negative.
b) One pair (dam and calf) and one cow.
c) One purchased in 2003 and four cows.

were all negative (No. 3–6, 11–15).

This study was carried out in collaboration with the Betsukai town office, which coordinated the five JAs. The 2 year PI surveillance and vaccination campaign covered all dairy farms each year and ended sometime near the end of the 2007 fiscal year with a farm level prevalence of 2.6% (23/881). The number of detected PI animals reduced from 28 to 13 over the course of two years, showing the effect of BTM screening/surveillance in eliminating PI cattle. However, it is possible there were more PI cattle, as PI would not be detected in cattle from which milk samples were not collected (i.e., cows that were dry or suffered from mastitis, etc.). Thus, BTM screening should be continued and repeated for at least a few more years. Also, heifers, 1,458 heads in 2006 and 1,658 in 2007, were tested for PI status prior to introduction of public breeding centers. Only one PI heifer was detected in each year, which also indicates a low PI incidence at the individual level. However, once BTM screening is completed in an area, it is essential to test the PI status in all animals that are newly introduced to a farm.

There were 23 farms in total that had at least one PI animal in either 2006 or 2007. In other words, PIs were not repeatedly detected at the same farm over the course of two years. However, only 15 farms (10 detected in 2006 and 5 in 2007) participated in the study (Table 2). The case control study is suitable for handling rare diseases [6]. In order to improve the precision of estimates, we chose 29 control farms, almost twice as many as the case farms [6]. There is no difference between the case and control farms in general farm management systems as well as some potential risk factors of BVDV infection, including the use of a public breeding center. Control farms were defined as farms where no PI cattle were detected between 2006 and 2007, including PI cattle found in individual blood tests done before entering public breeding centers. This does not mean that the control farms are completely free from PI, and we might have missed PI animals on a few farms due to cattle being excluded from the individual RT-PCR tests mentioned above. Further refining of the definition of control farms is required.

According to our univariate analysis, five factors were associated with the presence of PI cattle. Actually, these factors are not specific to BVDV infection but are general biosecurity-related factors. In the multivariate analysis, two factors, 1) no recent cattle purchase (between 2004 and 2007) and 2) no prevention of people/animals entering stables, were finally selected. Indeed, one of the important risk factors for BVDV infection, purchasing cattle, was negatively associated with the presence of PI cattle. This means that the case farms produced PI animals on their own farms, suggesting not only vertical transmission from dam to calf but also that indirect contact by people and animals plays an important role for disease transmission. Contact with BVDV-infected wild animals (e.g., deer) and indirect mechanical transmission through humans, agricultural machines and vehicles are also BVDV transmission routes. Prior to mass vaccination, we were sure that PI cattle were present at the case farms because of purchase of PI cows before 2004 and indirect transmission.

In our previous study based on data collected in Yubetsu town [10], we considered the following three factors as the main risk factors for quantitative risk assessment. The main transmission routes/venues (and duration of animal stay) are 1) purchase of animals (one day at a livestock market); 2) public breeding centers (5 months - May to October); and 3) livestock shows (3 days). In the present study, none of the above-mentioned risk factors were significant. The major difference between Yubetsu and Betsukai in terms of BVDV epidemiology is farm level prevalence, 28% [10] and 2.6% (23/881), respectively. Also, their distribution patterns of case farms are different; the case farms are distributed throughout the town in Yubetsu but are clustered in Betsukai. As we can see from the very low individual heifer level prevalence (0.7%=1/1458 in 2006), there are almost no BVDV PI cattle in the study population. Therefore, an indirect factor, no prevention of people/animals entering the premises, has a larger odds ratio, not direct factors such as purchase and show attendance. In a low prevalence area such as Betsukai, biosecurity-related factors might play a more important role in producing PI animals. For better understanding of BVDV infection in Betsukai, we need to conduct a further study including more case farms with extra information concerning clinical histories such as the presence of diarrhea at the individual animal level.

In conclusion, the prevalence and risk of BVDV infection in Betsukai is almost negligible. The identified risk factors are not specific to BVDV infection but are considered to be representative of the biosecurity status of the farms. Thus, it might be time to stop vaccination and establish BVDV systematic control as a sustainable strategy. Since BTM screening is relatively easy to launch, it could be a good measure for monitoring biosecurity status at the farm level in dairy farming areas where BVDV prevalence is as low as in Betsukai, Japan.

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REFERENCES

1. Anonymous 2006. EU Thematic network on control of BVDV position paper. http://bvdv-control.org/bilder/Position%20paper%20BVDV%20Control%20EU%20TN.pdf. (retrieved in June, 2009).
2. Betsukai town office 2009. Dairy cattle population in Betsukai in 2007. http://betsukai.jp/blog/ooo1/index.php?ID:412 (retrieved in November, 2009).
3. Brock, K.V., McCarthy, K., Chase, C.C.L. and Harland, R. 2006. Protection against Fetal Infection with Either Bovine

Viral Diarrhoea Virus Type 1 or Type 2 using a Noncytopathic Type 1 Modified-Live Virus Vaccine. *Vet. Ther.* **7**: 27–34.

4. Brownlie, J. 2002. Recent Developments and perspectives in bovine medicine. pp. 24–30. *In*: XXII World Buiatrics Congress, 2002 Aug. 18–23, Hannover, Germany.
5. Campbell, J.R. 2004. Effect of bovine viral diarrhea virus in the feedlot. *Vet. Clin. North Am. Food Anim. Pract.* **20**: 39–50.
6. Dohoo, I., Martin, W. and Stryhn, H. 2003. Case-control studies. pp. 163–175. *In*: Veterinary Epidemiologic Research, AVC, Prince Edward Island.
7. Grooms, D., Baker, J.C. and Ames, T.R. 2002. Diseases Caused by Bovine Diarrhea Virus. pp. 707–714. *In*: Large Animal Internal Medicine 3rd ed., (Bradford P.S. ed.), Mosby, St.Louis.
8. Gunn, G.J., Stott, A.W. and Humphry, R.W. 2004. Modelling and costing BVD outbreaks in beef herds. *Vet. J.* **167**: 143–149.
9. Gunn, G.J., Saatkamp, H.W., Humphry, R.W. and Stott, A.W. 2005. Assessing economic and social pressure for the control of bovine viral diarrhea virus. *Prev. Vet. Med.* **72**: 149–162.
10. Kadohira, M., Yasutomi, I., Markov, J. and Tajima, M. 2007. Quantitative risk assessment of Bovine Viral Diarrhea Virus Infection in Yubetsu, Hokkaido, Japan. *J. Vet. Epidemiol.* **11**: 32–39 (in Japanese).
11. Kim, S.G. and Dubovi, E.J. 2003. A novel simple one-step single-tube RT-duplex PCR method with an internal control for detection of bovine viral diarrhea virus in bulk milk, blood, and follicular fluid samples. *Biologicals* **31**: 103–106.
12. Moennig, V., Eicken, K., Flebbe, Frey, H.-R., Grummer, B., Haas, L., Greiser-Wilke, I. and Liess, B. 2005. Implementation of two-step vaccination in the control of bovine viral diarrhea (BVD). *Prev. Vet. Med.* **72**: 109–114.
13. National Livestock Breeding Center 2009. National cattle identification program. https://www.idnlbc.go.jp/top.html (retrieved in November 2009).
14. Sanderson, M.W. and Gnad, D.P. 2002. Biosecurity for reproductive disease. *Vet. Clin. North Am. Food Anim. Pract.* **18**: 79–98.
15. Siegwart, N., Hilbe, M., Hässig, M. and Braun, U. 2006. Increased risk of BVDV infection of calves from pregnant dams on communal Alpine pastures in Switzerland. *Vet. J.* **172**: 386–388.
16. Smith, D.R. and Grotelueschen, D.M. 2004. Biosecurity and biocontainment of bovine viral diarrhea virus. *Vet. Clin. North Am. Food Anim. Pract.* **20**: 131–149.
17. Yasutomi, I., Okazawa, M. and Hara, Y. 2004. Epidemiological investigation of Bovine Viral Diarrhea Virus infection with evaluating risk factors. *J. Vet. Epidemiol.* **8**: 77–83 (in Japanese).
18. Valle, P.S., Skjerve, E., Martin, S.W., Larssen, R.B., Østerås, O. and Nyberg, O. 2005. Ten years of bovine virus diarrhea virus (BVDV) control in Norway: a cost-benefit analysis. *Prev. Vet. Med.* **72**: 189–207.