NOTE *Virology*

Survey for detecting persistently infected cattle with bovine viral diarrhea in Japan

Ken-ichiro KAMEYAMA[1)], Misako KONISHI[1)], Toshiyuki TSUTSUI[1)] and Takehisa YAMAMOTO[1)]*

[1)]*Viral Diseases and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Kannondai 3–1–5, Tsukuba, Ibaraki 305–0856, Japan*

(Received 23 September 2015/Accepted 8 April 2016/Published online in J-STAGE 23 April 2016)

ABSTRACT. To establish effective and efficient control measures for bovine viral diarrhea (BVD) in Japan, a pilot survey on persistently infected (PI) animals in dairy farms was conducted. A total of 5,949 cattle from 79 farms in 11 prefectures were tested; seven cattle in six farms were identified as PI animals. The proportion of farms with PI animals in Japan was calculated as 7.6% (95% confidence interval: 3.1–16.4%), and proportion of cattle tested as PI animals was 0.12% (95% confidence interval: 0.05–0.25%). The presence of only one or two animals in PI positive farms suggested the application of screening tests covering almost all cattle in each farm using pooled serum or bulk milk could be effective for implementing a large-scale survey for detecting PI animals.

KEY WORDS: bovine viral diarrhea, dairy farm, Japan, persistent infection, surveillance

doi: 10.1292/jvms.15-0555; *J. Vet. Med. Sci.* 78(8): 1329–1331, 2016

Bovine viral diarrhea (BVD) is a viral disease that affects cattle productivity and may cause abortion, diarrhea and mucosal disorders [3]. When pregnant cattle are infected during the early stage of gestation, some of their fetuses become immunologically tolerant to the BVD virus and are born as persistently infected (PI) calves [8]. The PI animals often show mild clinical signs such as respiratory symptoms caused by opportunistic infections, resulting in poor productivity, whereas the mortality of PI animals is not so high [3]. PI animals shed large amounts of BVD viruses in their entire lives. Therefore, they play an important role in virus spread within and between farms [9, 10]. Although the BVD virus has spread in many countries worldwide, Sweden and other Nordic countries have achieved eradication or significant reduction in the prevalence of BVD using control measures, such as test and slaughter of PI animals [11, 13].

In Japan, BVD was designated as a notifiable disease in 1998. Since then, the number of reported cases has constantly been 100–200 animals every year. Even though any clear definition was not established for cases to be reported, interview with official veterinarians in prefectures indicated that these reported cases are not a transiently infected (acute) cattle but PI cattle, in general.

Considering the importance of PI animals on BVD spread and control [2], the presence of PI animals in a cattle population should be evaluated to establish effective and efficient control measures. In Japan, both modified live vaccines and killed vaccines are available for protecting cattle from BVDV infection. Since most beef cattle markets in which fattening calves mainly traded require BVD vaccination before the entry of animals, vaccination coverage is considered higher in beef cattle than in dairy cattle. In this sense, presence of PI animals in beef cattle population is presumed to be influenced by BVD vaccine. Therefore, we focused on dairy cattle population as a target of this study.

In this study, we conducted a pilot survey of PI animals in dairy farms to explore appropriate surveillance methods for detecting these animals on a larger scale. Twelve prefectures, located in Kanto and westward regions, agreed to participate in the survey that was performed in 2014. The funding was provided by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan. Each of the 12 prefectural governments selected a maximum of 10 dairy farms and conducted antigen detection tests on all cattle, preferably with unvaccinated cattle, as much as possible. The data from one prefecture which tested seven farms were removed from this study, because relatively small proportion of animals was tested in these farms. The tests were performed by RT–PCR using 324–326 primers [14] or ELISA (BVDV Ag ELISA Kit; IDEXX Laboratories, Tokyo, Japan) at the livestock hygiene service centers of each prefecture. Both method was assumed to have enough sensitivity to detect PI animals, because PI animals express large amount of BVDV in their brood [4, 7].

To distinguish between PI and acute infection, animals showing positive results were re-tested at intervals of at least 2 weeks. Animals that tested positive on both tests were defined as PI, whereas animals with negative results in the second test were designated as having an acute infection. Concurrently, information on the farm size, barn type, history of introducing cattle into the farm and vaccination history was investigated. From the results of the survey, the proportion of PI animals was estimated. Additionally, in farms where PI animals were detected, antigen tests using bulk milk and/or pool sera of all cattle within the farm were implemented to examine the applicability of these pooled sampling techniques for screening purposes. For pooled sera PCR, 20–50 μl of each serum were collected to a vessel. Vi-

*CORRESPONDENCE TO: YAMAMOTO, T., Viral Diseases and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Kannondai 3–1–5, Tsukuba, Ibaraki 305–0856, Japan. e-mail: mtbook@affrc.go.jp

ral RNA was extracted from an aliquot of these pooled sera. For bulk milk PCR, 50 m*l* of bulk milk were centrifuged at 3,000 rpm for 20 min, and then, pelleted somatic cells were washed with PBS and re-pelleted. All pelleted cells were used for RNA extraction. The viral RNAs were applied for RT-PCR using primer set 324–326 [14].

As a result, a total of 5,949 cattle kept in 79 farms in 11 prefectures were included in this study. Of the 79 farms included in this study, 70 (88.6%) had dairy cattle only, whereas the other nine (11.4%) kept both dairy and beef cattle. The proportion of tested animals in total was 89.5% (5,949/6,646). The proportions of animals tested in the 79 farms ranged from 61.4% to 100%. In 45 of 70 dairy and 5 of 9 mixed farms, all animals were tested. The proportion of farms with 50 or more animals per farm (67.1% 53/79) was greater than that of all dairy farms in Japan (37.4%). As shown in Table 1, over 99% of tested animals were dairy breeds, including Holstein-Friesian (HF) and Jersey. The proportions of Japanese Black (JB) and Crossbred were under 1%. There were nine cattle with positive results on the first testing; among them, seven were PI animals, and two were considered as having an acute infection (Table 2). Among these 6 farms with PI animals, two had vaccination history within a year from the test, whereas the other four didn't. There were no farms wherein both PI and acute infection were observed. Among the seven PI animals, two (JB and HF) were kept in the same farm (farm No.5), while the other five were kept in five different farms. Accordingly, the proportion of farms with PI animals was 7.59% (6/79; 95% confidence interval, 3.1–16.4%), and the proportion of PI animals was 0.12% (7/5, 949; 95% confidence interval, 0.05–0.25%).

The age of the seven PI animals at sampling ranged from 2.1 to 34.8 months; six animals under 17 months old were considered as heifers.

All PI animals were born in their own farms and had no history of being kept in the other farms. In this survey, no information on their dams was obtained. These seven PI animals were not vaccinated. This may be due to the fact that farms with unvaccinated cattle were primarily selected in this study. Among the PI animals, six were HF, and one was JB; this JB calf was born from an HF dam by embryo transfer. Therefore, infection of the HF dam during gestation was considered to have caused immunological tolerance of this JB cow to the BVD virus. In the three of six farms where PI animals were detected, RT–PCR using pooled sera of almost all cattle (73/73, 69/69 and 48/49), including those of the PI cattle, was conducted. All of these three pooled samples showed positive results. In addition, for the farm with 69 cattle, PCR of the bulk milk from 57 cows, including one PI cow, tested positive.

In the present study, the proportion of PI cattle in dairy farms was revealed for the first time in Japan by large-scale sampling. In other countries, the proportion of PI animals among dairy cattle was reported to be 0.9%–53% at the farm level and 0.11%–1.4% at the animal level [1, 5, 6, 12]. The observed proportion of PI animals in this study was within these values but rather lower. However, caution should be exercised when these results are interpreted. The tested farms in this study were not randomly selected; therefore, our results may be biased.

Table 1. Breed and number of cattle analyzed for PI with BVDV

Breed	No. of animals	%	No. of PI's
Holstein-Friesian	5,806	97.6	6
Jersey	105	1.8	0
Crossbred	28	0.5	0
Japanese Black	4	0.1	1
Others	6	0.1	0
Total	5,949	100	7

PI, Persistently infected; BVD, Bovine viral diarrhea.

Table 2. Characteristics of the detected PI cattle with BVDV

Farm	Sex	Usage	Breed	Movement between farms	Age (mo)	Vaccination
1	F	Daily	HF	No	2.3	No
2	F	Daily	HF	No	34.8	No
3	F	Daily	HF	No	12.0	No
4	F	Daily	HF	No	16.2	No
5	F	Meat	JB	No	2.3	No
5	F	Daily	HF	No	15.2	No
6	F	Daily	HF	No	2.1	No

PI, persistently infected; BVDV, bovine viral diarrhea virus; HF, Holstein-Friesian; JB, Japanese Black.

In this study, although antigen tests were performed for most animals kept in farms, only one PI animal was detected in five out of six farms. Therefore, the proportion of PI animals in a farm was very low, even if PI animals existed. These results imply that when a survey is planned to reliably detect PI animals in a population, it is necessary to test all animals in each farm. Although most of the PI animals detected in this study were heifers, it is not reasonable to expect the absence of PI animals among older cows and to remove aged cattle to be tested when planning a surveillance to detect PI animals in future.

However, it would be costly and time-consuming to subject all cattle on a farm to antigen detection using ELISA or RT–PCR. Therefore, tests using pooled samples could be a practical alternative. In this study, even examined in only three farms, a positive result was obtained by RT–PCR of pooled sera and bulk milk from a relatively large number of cattle that included one PI animal. This result was consistent with the result of Weinstock *et al.* which reported that antigen detection was possible using pooled samples even if the sources included only one PI animal and 99 negatives [15].

The results of our study provide indispensable information for further consideration of BVD control strategies in Japan.

ACKNOWLEDGMENTS. This research was performed as part of the BVD surveillance project of the Ministry of Agriculture, Forestry and Fisheries. We deeply thank all organizations concerned in this project.

REFERENCES

1. Braun, U., Schönmann, M., Ehrensperger, F., Hilbe, M., Brunner, D., Stärk, K. D. and Giger, T. 1998. Epidemiology of bovine virus diarrhoea in cattle on communal alpine pastures in Switzerland. *Zentralbl. Veterinarmed. A* **45**: 445–452. [Medline] [CrossRef]
2. Graham, D. A., Clegg, T. A., Thulke, H. H., O'Sullivan, P., McGrath, G. and More, S. J. 2016. Quantifying the risk of spread of bovine viral diarrhoea virus (BVDV) between contiguous herds in Ireland. *Prev. Vet. Med.* **126**: 30–38. [Medline] [CrossRef]
3. Groomes, D., Baker, J. C. and Ames, T. R. 2002. Diseases caused by bovine virus diarrhea virus. pp. 707–714. *In*: Large Animal Internal Medicine, 3rd ed. (Smith, B. ed.), Mosby, St. Louis.
4. Hilbe, M., Stalder, H., Peterhans, E., Haessig, M., Nussbaumer, M., Egli, C., Schelp, C., Zlinszky, K. and Ehrensperger, F. 2007. Comparison of five diagnostic methods for detecting bovine viral diarrhea virus infection in calves. *J. Vet. Diagn. Invest.* **19**: 28–34. [Medline] [CrossRef]
5. Houe, H. and Meyling, A. 1991. Prevalence of bovine virus diarrhoea (BVD) in 19 Danish dairy herds and estimation of incidence of infection in early pregnancy. *Prev. Vet. Med.* **11**: 9–16. [CrossRef]
6. Houe, H., Baker, J. C., Maes, R. K., Wuryastuti, H., Wasito, R., Ruegg, P. L. and Lloyd, J. W. 1995. Prevalence of cattle persistently infected with bovine viral diarrhea virus in 20 dairy herds in two counties in central Michigan and comparison of prevalence of antibody-positive cattle among herds with different infection and vaccination status. *J. Vet. Diagn. Invest.* **7**: 321–326. [Medline] [CrossRef]
7. Masuda, T., Ashiba, T., Yamasato, H. and Kameyama, K. 2016. Verification of a new commercial antigen-specific ELISA for detection of Bovine Viral Diarrhea Virus. *J. Jpn. Vet. Med. Assoc.* (in Press).
8. McClurkin, A. W., Littledike, E. T., Cutlip, R. C., Frank, G. H., Coria, M. F. and Bolin, S. R. 1984. Production of cattle immunotolerant to bovine viral diarrhea virus. *Can. J. Comp. Med.* **48**: 156–161. [Medline]
9. Moennig, V. and Plagemann, P. G. 1992. The pestiviruses. *Adv. Virus Res.* **41**: 53–98. [Medline] [CrossRef]
10. Nettleton, P. F. and Entrican, G. 1995. Ruminant pestiviruses. *Br. Vet. J.* **151**: 615–642. [Medline] [CrossRef]
11. Sandvik, T. 2004. Progress of control and prevention programs for bovine viral diarrhea virus in Europe. *Vet. Clin. North Am. Food Anim. Pract.* **20**: 151–169. [Medline] [CrossRef]
12. Schreiber, P., Dubois, F., Drèze, F., Lacroix, N., Limbourg, B. and Coppe, P. 1999. Prevalence of bovine virus diarrhoea virus infection in Belgian white blue cattle in southern Belgium. *Vet. Q.* **21**: 28–32. [Medline] [CrossRef]
13. Ståhl, K. and Alenius, S. 2012. BVDV control and eradication in Europe—an update. *Jpn. J. Vet. Res.* **60** Suppl: S31–S39. [Medline]
14. Vilcek, S., Herring, A. J., Herring, J. A., Nettleton, P. F., Lowings, J. P. and Paton, D. J. 1994. Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. *Arch. Virol.* **136**: 309–323. [Medline] [CrossRef]
15. Weinstock, D., Bhudevi, B. and Castro, A. E. 2001. Single-tube single-enzyme reverse transcriptase PCR assay for detection of bovine viral diarrhea virus in pooled bovine serum. *J. Clin. Microbiol.* **39**: 343–346. [Medline] [CrossRef]