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NOTE

Virology

Endemic infections of bovine viral diarrhea virus genotypes 1b and 2a isolated from cattle in Japan between 2014 and 2020

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ABSTRACT. Bovine viral diarrhea virus (BVDV) is a causative agent of bovine viral diarrhea. In Japan, a previous study reported that subgenotype 1b viruses were predominant until 2014. Because there is little information regarding the recent epidemiological status of BVDV circulating in Japan, we performed genetic characterization of 909 BVDV isolates obtained between 2014 and 2020. We found that 657 and 252 isolates were classified as BVDV-1 and BVDV-2, respectively, and that they were further subdivided into 1a (35 isolates, 3.9%), 1b (588, 64.7%), 1c (34, 3.7%), and 2a (252, 27.7%). Phylogenetic analysis using entire E2 coding sequence revealed that a major domestic cluster in Japan among BVDV-1b and 2a viruses were unchanged from a previous study conducted from 2006 to 2014. These results provide updated information concerning the epidemic strain of BVDV in Japan, which would be helpful for appropriate vaccine selection.

KEY WORDS: bovine viral diarrhea virus, genotype, Japan, phylogenetic analysis

Bovine viral diarrhea (BVD) is a disease characterized by fever, diarrhea, respiratory symptoms, and reproductive disorders, and is caused by infection with bovine viral diarrhea virus (BVDV), which belongs to the genus *Pestivirus* within the family *Flaviviridae*. BVDV is divided into two biotypes, cytopathogenic and non-cytopathogenic, by its pathogenic properties in certain cultured cells [14], and intrauterine infection with non-cytopathogenic BVDV during bovine gestation results in the production of persistently infected (PI) calves [13]. Because PI calves shed large amounts of virus throughout their lives, they become the main source of ongoing infection among susceptible herds. According to a Japanese survey of PI animals in dairy farms located in Kanto and westward regions, the proportion of farms with PI was 7.6%, and the proportion of cattle tested as PI was 0.12% [10]. Other groups reported that the proportion of PI in dairy and beef farms in the Tohoku region was 0.38% [6], and that in beef farms in the Kyushu region was 0.18% [2]. Although the annual number of BVD diagnoses that are mandated to be notified to the government was approximately 300−400 over the last five years [17], it is suggested that a large number of hidden PIs exist in Japan because their estimated number are calculated at more than 4,500 from the reported proportion (0.12%) [10] and total number of cattle in Japan (approximately 3.9 million head in 2020) [21].

To reduce economic losses caused by BVD, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of the Japanese government formulated guidelines for prevention and control measures against BVDV in 2016 [16]. These guidelines encourage the implementation of a screening program for detecting PI animals using a bulk-tank milk test, spot test, or pooled serum test as an annual routine inspection and a test for newly introduced cattle, and recommend carrying out BVDV vaccination, a test before entering common pastures and stamping out any detected PI. However, the states of implementation of surveillance and countermeasures for BVD differ among Japanese domestic regions; therefore, it is important to establish suitable measures to control BVD for each situation.

BVDV is genetically categorized into genotypes 1 (BVDV-1) and 2 (BVDV-2) [31]. BVDV-1 can be further divided into at least 21 subgenotypes (1a−1u), while BVDV-2 can be divided into four subgenotypes (2a−2d) [33] based on nucleotide sequences, such as the 5′-untranslated region (5′UTR) and E2. E2 is known as an envelope protein of BVDV which is the major target of neutralizing antibodies [5]. In Japan, BVD outbreaks have been reported since the 1960s [25], and although at the beginning of the outbreaks the isolates were only of the BVDV-1 genotype, the first isolation of BVDV-2 was reported in 1998 [18]. According

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to epidemiological surveys conducted until the 1990s, the number of reported isolates of BVDV-1a viruses was comparable to or slightly higher than that of BVDV-1b [19, 29]. On the other hand, during the last two decades, several studies have reported that BVDV-1b viruses are predominant in Japan and that the number of BVDV-1a isolates tended to decrease, while BVDV-2a outbreaks became more frequent [1, 12]. Because antigenicity differs among BVDV subgenotypes [1, 20], the latest information concerning genotypes of epidemic strains in Japan is quite important for appropriate selection of vaccines. Although commercially available vaccines targeting BVDV-1 have been limited to those containing only BVDV-1a strains for a long period in Japan, an inactivated vaccine containing BVDV-1b strains has become available for use since 2014. Since there is little information regarding the epidemic status of BVDV circulation in Japan over the last 6 years, in the present study, we reported genetic characterization of BVDVs isolated from cattle between 2014 and 2020, which is important in vaccination efforts.

Serum samples and BVDV isolates from PI animals or a small number of transiently infected animals that were diagnosed in farms between 2014 and 2020 were kindly provided by Japanese local livestock hygiene service centers. A total of 909 isolates were collected during the study period: 734 isolates from Hokkaido, 73 from Gunma, 19 from Ibaraki, 5 from Gifu, 8 from Hyogo, 28 from Okayama, 13 from Kumamoto, and 29 isolates from Oita (Supplementary Fig. 1).

Viral RNA was extracted using TRIzol LS (Thermo Fisher Scientific, Waltham, MA, USA) or Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. PCR amplification and sequence analysis were conducted several times in the two institutes; each method is separately described in the Supplementary Materials and Methods. Multiple sequence alignments were produced by the ClustalW algorithm [32] with default parameters using GENETYX v.14 software (Genetyx Corp., Tokyo, Japan). Phylogenetic analysis based on viral 5′-UTR and E2 sequences was carried out using the maximum-likelihood method with 1,000 bootstrap replicates using MEGA X software [11]. The optimal models for phylogenetic analysis were determined by using the "Find Best DNA/Protein Models" function in MEGA software. The "complete deletion" was selected for Gaps/Missing Data Treatment.

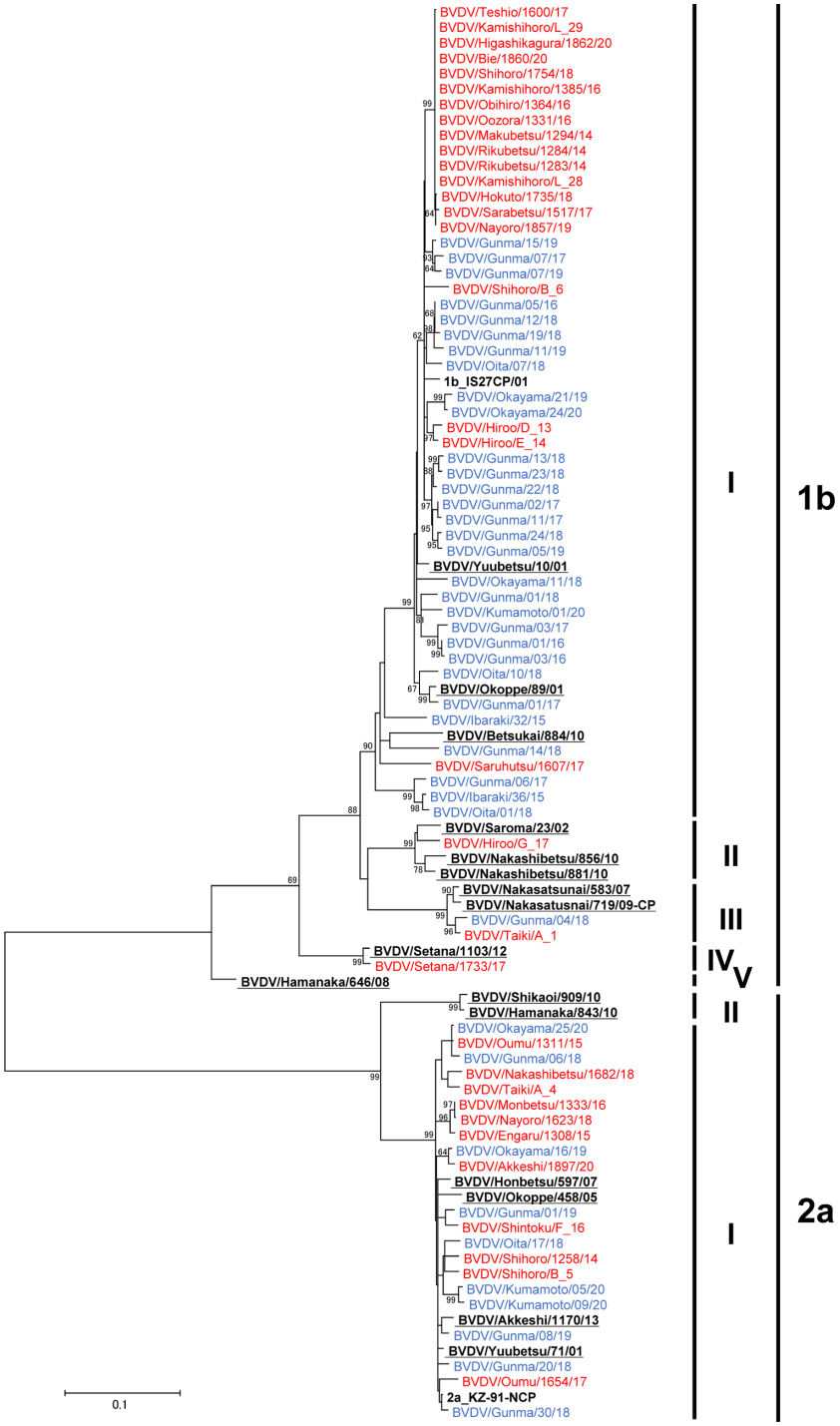
Genotyping of 909 BVDV isolates obtained between 2014 and 2020 in Japan was conducted based on 5′-UTR nucleotide sequences, revealing that they were divided into 657 BVDV-1 isolates and 252 BVDV-2 isolates (Table 1). BVDV-1 was further subdivided into BVDV-1a (35 isolates, 3.9%), 1b (588, 64.7%), and 1c (34, 3.7%), while all BVDV-2 isolates were classified as BVDV-2a (252, 27.7%). With regard to regional differences, comparison of the genotypic proportion between Hokkaido and the other prefectures showed similar variations in subgenotypes during our study period, which suggested that there were no clear differences between these regions (Supplementary Fig. 2).

Fifty-three isolates of BVDV-1b and 21 isolates of BVDV-2a were selected from several sampling areas and the complete E2 encoding sequences were determined for more detailed characterization of the representative isolates. Phylogenetic analysis of these selected isolates was conducted based on both 5′-UTR and E2 sequences, indicating that the subgenotype classification based on the E2 gene was completely consistent with the results of genotyping based on 5′-UTR sequences (Fig. 1, Supplementary Fig. 3). According to a previous report, Japanese BVDV-1b and 2a isolates are branched into five and two clusters, respectively, by E2-based phylogenetic analysis [1]. Analysis of the isolates obtained in this study indicated that BVDV-1b cluster I and BVDV-2a cluster I were the major isolates among each subgenotype, which was consistent with a similar distribution indicated in a previous report (Fig. 1) [1].

The epidemiological status of BVDV circulation in Japan has been reported continuously from 1974 to 1999 involving several prefectures [19], from 2000 to 2006 in several prefectures [12], and from 2006 to 2014 in Hokkaido [1]. According to previous reports over the last two decades [1, 12], BVDV-1b viruses were predominant in Japan, although a different survey based on neutralizing antibody titers indicated that cattle producing antibodies specific for BVDV-1a were predominant among unvaccinated cattle [15]. In the present study, we provided updated genetic information on Japanese BVDV isolates from 2014 to 2020 in several prefectures and showed that BVDV-1b was still the predominant subgenotype and that BVDV-2a was the second major isolate, in agreement with previous reports (Table 1) [1, 12]. Additionally, phylogenetic analysis using E2 coding sequences showed that the major clusters among each subgenotype were the same as those reported from 2006 to 2014 (Fig. 1) [1]. These results may suggest poor changes in BVDV epidemic situations and less progression for disease elimination in Japan; in particular, the number of BVD notifications has remained at the same level over recent years [17]. However, it is difficult to discuss the actual changes in prevalence in the current Japanese state because of our study design that collected only

Table 1. Genotyping of all isolates collected in this study from 2014 to 2020

Year of isolation	Prefecture	No. of sample	Genotype			
			BVDV-1			BVDV-2
			1a	1b	1c	2a
2014	Hokkaido	66	4	33	6	23
	Ibaraki	1	0	0	1	0
2015	Hokkaido	76	7	34	11	24
	Ibaraki	10	0	8	2	0
2016	Hokkaido	147	4	120	5	18
	Gunma	10	0	9	0	1
	Gifu	1	0	1	0	0
2017	Hokkaido	156	7	95	0	54
	Ibaraki	2	1	0	1	0
	Gunma	13	0	13	0	0
	Hyogo	8	0	4	0	4
2018	Hokkaido	148	9	91	6	42
	Ibaraki	6	0	5	0	1
	Gunma	31	0	22	0	9
	Gifu	1	0	1	0	0
	Okayama	13	0	10	0	3
	Kumamoto	2	0	2	0	0
	Oita	26	0	22	0	4
2019	Hokkaido	84	0	55	1	28
	Gunma	19	0	11	0	8
	Gifu	2	0	2	0	0
	Okayama	9	0	6	0	3
	Oita	3	0	0	0	3
2020	Hokkaido	57	2	39	1	15
	Gifu	1	0	0	0	1
	Okayama	6	0	3	0	3
	Kumamoto	11	1	2	0	8
Total		909	35	588	34	252



BVDV-positive samples from each region. Thus, further research will be required to determine the actual prevalence of BVD. Here, it should be noted that an increase or decrease in the number of BVDV isolates during every study year did not always reflect the results of BVD outbreaks, but were mainly affected by active surveillance programs conducted involving farms and local veterinarian offices.

During our study period, there were no clear regional differences in the proportion of viral subgenotypes between isolates from Hokkaido and those from other prefectures (Supplementary Fig. 2). This finding implies a similar epidemic state in Hokkaido and other prefectures and therefore suggests that national BVDV circulation is accompanied by cattle movement. In Switzerland, where BVD has been successfully controlled, animal movements such as summer pasturing and cattle purchases are reported to be an important risk factor for BVDV transmission and subsequent birth of PI calves [27], as well as in Japan [4]. Although there are few detailed reports concerning cattle movements in Japan, it is assumed that the movement of cattle involved in Hokkaido prefecture is very frequent because Hokkaido is a major production region for domestic cattle, which may result in a similar distribution of viral genotypes among the regions. Our results suggest that domestic animal movement is still the main cause of BVDV circulation in Japan; thus, we need to focus more on the inspection systems for testing of newly introduced, purchased, and pastured cattle.

To prevent PI calves from being born on farms, appropriate vaccination is important, as is the viral antigen testing of calves and newly introduced cattle. Currently, the following vaccine products are commercially available in Japan: one product of attenuated live vaccine containing BVDV-1a only, one product of attenuated live vaccine containing BVDV-1a and 2a, three products of inactivated vaccine containing BVDV-1a and 2a, and one product of inactivated vaccine containing BVDV-1b and

Fig. 1. Phylogenetic tree of 1b and 2a isolates reported in this study based on complete E2 coding sequences. Nucleotide sequence of the entire E2 gene, which corresponds to partial sequences of the IS27CP/01 strains (accession no. AB359935; positions 1−1,122), of 53 bovine viral diarrhea virus (BVDV)-1b isolates and 21 BVDV-2a isolates were used for phylogenetic analysis. Multiple alignments were produced by the ClustalW algorithm with default parameters using GENETYX v.14 software (Genetyx Corp., Tokyo, Japan). The phylogenetic tree was constructed using the maximum-likelihood method (Tamura 3-parameter, gamma distributed with invariant sites) and bootstrap analysis (n=1,000) using MEGA X software [11]. All accession numbers of the field isolates presented in the phylogenetic tree are listed in Supplementary Table 1. Reference strains are indicated in bold and the isolates described in a previous study [1] for cluster classification (five clusters for BVDV-1b, I–V; two clusters for BVDV-2a, I–II) are underlined. GenBank accession numbers of the reference strains are as follows: IS27CP/01 (AB359935), BVDV/Yuubetsu/10/01 (AB896805), BVDV/Okoppe/89/01 (AB896807), BVDV/Betsukai/884/10 (AB896811), BVDV/Saroma/23/02 (AB896806), BVDV/Nakashibetsu/856/10 (AB896810), BVDV/Nakashibetsu/881/10 (AB894349), BVDV/Nakasatsunai/583/07 (AB896808), BVDV/Nakasatusnai/719/09-CP (AB896809), BVDV/Setana/1103/12 (LC016732), BVDV/Hamanaka/646/08 (LC016731), BVDV/Shikaoi/909/10 (LC016728), BVDV/Hamanaka/843/10 (LC016727), BVDV/Okoppe/458/05 (LC016724), BVDV/Honbetsu/597/07 (LC016725), BVDV/Yuubetsu/71/01 (LC016723), BVDV/Akkeshi/1170/13 (LC016726) and KZ-91-NCP (LC649064). The field isolates for Hokkaido prefecture are indicated in red, while those in other prefectures are shown in blue.

2a [23], all of which are multivalent vaccines and include other viral antigens related to bovine respiratory diseases. In this study, the proportion of BVDV-1a isolates was 3.9% (Table 1), which is lower than that reported in previous studies, 21.3% (2000−2006) [12] and 12.1% (2006−2014) [1]. Therefore, it may be possible that a decrease in the proportion of BVDV-1a observed recently suggests success in preventing viral infection by vaccination. However, more importantly, as a previous report revealed that BVDV-1a showed higher pathogenicity than 1b [1], PI cattle infected with BVDV-1a are more easily diagnosed and culled, preventing the spread of viruses on farms. On the other hand, the proportion of BVDV-1b and 2a isolates did not exhibit a large departure from previous studies [1, 12], and the major clusters of these subgenotypes by phylogenetic analysis were also comparable (Fig. 1). For this reason, it is less likely that circulating BVDV-1b and 2a in Japan have escaped vaccine pressure. Although there are no accurate data on vaccine coverage against BVDV in Japan, the domestic sales of BVDV vaccines calculated by the database are approximately 2 million doses per year from 2012 to 2018 (Supplementary Table 2) [22]. If inactivated vaccines are used for double administration of vaccination programs, the vaccine coverage in Japan is estimated at around 34.4−40.7%; however, in the situation of triple-administrations or combined use of live-attenuated and inactivated vaccines, the actual vaccine coverage may be possibly lower. Because we do not have sufficient information surrounding vaccination records of PI animals or their dams in the present study, there are limitations in discussing the possibilities of vaccine escape. However, the cross-reactivity of antisera against BVDV-1a was 8- to 32-fold less active against BVDV-1b in virus neutralization assays [1, 20]; therefore, it is possible that BVDV-1b viruses are minimally affected by vaccine pressure in the current Japanese situation. In Nordic countries, BVD control strategies using antibody tests of bulk-tank milk samples have been successful without vaccination [7]. In contrast, since all available vaccines for BVDV in Japan are multivalent and users do not stop only BVDV vaccination, it is difficult for the Nordic strategies to be simply applied to the current Japanese domestic situation. Thus, in Japan, we need to consider the BVD control program including vaccination and to use vaccines appropriately selected in accordance with a national epidemic status, because of the possibility of low cross-reactivity among different genotype viruses [1, 20].

Now, the states involved in countermeasures for BVD (e.g., number of livestock, status of vaccine coverage, or capacity for implementation of inspections) differ across Japanese domestic regions; therefore, it is important to select suitable ways for each prefecture based on the situation in that area. For domestic model cases for controlling BVD, large-scale mass vaccination and test and culling programs were implemented in Nemuro region of Hokkaido prefecture [9, 28]. However, because the current BVDV vaccine cannot completely prevent intrauterine infection [24], it should be notified that BVD control by vaccination alone will be less effective. In addition, several studies using simulation models indicated that testing and culling of BVDV positive animals reduce BVD prevalence more effectively than vaccination alone [8, 30]. Hence, the BVD control program under the current Japanese situation should focus on certain diagnoses and stamping out of PI cattle by active surveillance with low sampling costs, followed by optional vaccination when cattle are exposed to an elevated risk of BVDV infection. Now, several studies have reported surveillance programs to detect PI animals by labor-saving methods: for example, screening tests using bulk-tank milk and milk tanker samples in Ibaraki prefecture where BVDV vaccines were not used on most dairy farms [3]. If there is sufficient capacity for inspection, implementation of individual testing for viral antigens at scenes of cattle movement, such as the common pasture and auction marketing of calves, is recommended to further reduce the prevalence of BVD. Because testing and culling of all newborn calves will incur very considerable costs [26], long-term and nationwide support for BVD eradication programs is essential for success.

To control BVD, we need to consider various actions suitable for each region's status; therefore, both appropriate vaccine selection and optimization of surveillance programs are important. In the present study, we provided epidemiological information concerning recent epidemic strains of BVDV in Japan, which will be helpful in selecting future vaccine strains effective for disease control. Continuous surveillance to reveal the genetic distribution of BVDVs will be required to evaluate future vaccine effectiveness.

APPENDIX

As for the availability of data, the 5′-UTR and E2 nucleotide sequences of 74 isolates described in Fig. 1 and Supplementary Fig. 3 were submitted to DDBJ/EMBL/GenBank and their accession numbers are listed in Supplementary Table 1. The alignment data of those phylogenetic trees are provided as Supplementary Data 1 and 2. For other strains, the data which support the results of genotyping in this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

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REFERENCES

1. Abe, Y., Tamura, T., Torii, S., Wakamori, S., Nagai, M., Mitsuhashi, K., Mine, J., Fujimoto, Y., Nagashima, N., Yoshino, F., Sugita, Y., Nomura, T., Okamatsu, M., Kida, H. and Sakoda, Y. 2016. Genetic and antigenic characterization of bovine viral diarrhea viruses isolated from cattle in

Hokkaido, Japan. *J. Vet. Med. Sci.* **78**: 61–70. [Medline] [CrossRef]
2. Agah, M. A., Notsu, K., El-Khaiat, H. M., Arikawa, G., Kubo, M., Mitoma, S., Okabayashi, T., Mekata, H., Elhanafy, E., El Daous, H., Mai, T. N., Nguyen, T. H., Isoda, N., Sakoda, Y., Norimine, J. and Sekiguchi, S. 2019. Slaughterhouse survey for detection of bovine viral diarrhea infection among beef cattle in Kyushu, Japan. *J. Vet. Med. Sci.* **81**: 1450–1454. [Medline] [CrossRef]
3. Akagami, M., Takayasu, M., Ooya, S., Kashima, Y., Tsuzuku, S., Ootani, Y., Ouchi, Y. and Hayama, Y. 2020. Screening of persistently infected cattle with bovine viral diarrhea virus on dairy farms by using milk tanker and bulk tank milk samples for viral RNA and viral-specific antibody detection. *J. Vet. Med. Sci.* **82**: 607–614. [Medline] [CrossRef]
4. Akagami, M., Seki, S., Kashima, Y., Yamashita, K., Oya, S., Fujii, Y., Takayasu, M., Yaguchi, Y., Suzuki, A., Ono, Y., Ouchi, Y. and Hayama, Y. 2020. Risk factors associated with the within-farm transmission of bovine viral diarrhea virus and the incidence of persistently infected cattle on dairy farms from Ibaraki prefecture of Japan. *Res. Vet. Sci.* **129**: 187–192. [Medline] [CrossRef]
5. Deregt, D., Bolin, S. R., van den Hurk, J., Ridpath, J. F. and Gilbert, S. A. 1998. Mapping of a type 1-specific and a type-common epitope on the E2 (gp53) protein of bovine viral diarrhea virus with neutralization escape mutants. *Virus Res.* **53**: 81–90. [Medline] [CrossRef]
6. Fukunari, K., Yaegashi, G., Chiba, S. and Kameyama, K. 2013. Surveillance of bovine viral diarrhea virus infection in dead cattle in Iwate prefecture. *Nippon Juishikai Zasshi* **66**: 785–790 (in Japanese).
7. Houe, H., Lindberg, A. and Moennig, V. 2006. Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. *J. Vet. Diagn. Invest.* **18**: 427–436. [Medline] [CrossRef]
8. Isoda, N., Asano, A., Ichijo, M., Wakamori, S., Ohno, H., Sato, K., Okamoto, H., Nakao, S., Kato, H., Saito, K., Ito, N., Usui, A., Takayama, H. and Sakoda, Y. 2017. Evaluation of control measures for bovine viral diarrhea implemented in Nemuro District, Hokkaido, Japan, using a scenario tree model. *J. Vet. Med. Sci.* **79**: 1172–1181. [Medline] [CrossRef]
9. Kadohira, M. and Tajima, M. 2010. A case control study of bovine viral diarrhea virus (BVDV) persistent infection (PI) in Betsukai, Hokkaido, Japan. *J. Vet. Med. Sci.* **72**: 635–638. [Medline] [CrossRef]
10. Kameyama, K., Konishi, M., Tsutsui, T. and Yamamoto, T. 2016. Survey for detecting persistently infected cattle with bovine viral diarrhea in Japan. *J. Vet. Med. Sci.* **78**: 1329–1331. [Medline] [CrossRef]
11. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**: 1547–1549. [Medline] [CrossRef]
12. Matsuno, K., Sakoda, Y., Kameyama, K., Tamai, K., Ito, A. and Kida, H. 2007. Genetic and pathobiological characterization of bovine viral diarrhea viruses recently isolated from cattle in Japan. *J. Vet. Med. Sci.* **69**: 515–520. [Medline] [CrossRef]
13. 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]
14. McClurkin, A. W., Bolin, S. R. and Coria, M. F. 1985. Isolation of cytopathic and noncytopathic bovine viral diarrhea virus from the spleen of cattle acutely and chronically affected with bovine viral diarrhea. *J. Am. Vet. Med. Assoc.* **186**: 568–569. [Medline]
15. Minami, F., Nagai, M., Ito, M., Matsuda, T., Takai, H., Jinkawa, Y., Shimano, T., Hayashi, M., Seki, Y., Sakoda, Y., Sugiura, K. and Akashi, H. 2011. Reactivity and prevalence of neutralizing antibodies against Japanese strains of bovine viral diarrhea virus subgenotypes. *Comp. Immunol. Microbiol. Infect. Dis.* **34**: 35–39. [Medline] [CrossRef]
16. Ministry of Agriculture Forestry and Fisheries (MAFF). 2016. Guidelines for Prevention and Control of Bovine Viral Diarrhea and Mucosal Disease. http://www.maff.go.jp/j/ syouan/douei/pdf/bvd_md_gl.pdf [accessed on March 3, 2021] (in Japanese).
17. Ministry of Agriculture Forestry and Fisheries (MAFF). 2020. Annual Statistics of Domestic Animal Infectious Diseases. http://www.maff.go.jp/j/ syouan/douei/kansi_densen/kansi_densen.html [accessed on March 3, 2021] (in Japanese).
18. Nagai, M., Sato, M., Nagano, H., Pang, H., Kong, X., Murakami, T., Ozawa, T. and Akashi, H. 1998. Nucleotide sequence homology to bovine viral diarrhea virus 2 (BVDV 2) in the 5′ untranslated region of BVDVs from cattle with mucosal disease or persistent infection in Japan. *Vet. Microbiol.* **60**: 271–276. [Medline] [CrossRef]
19. Nagai, M., Ito, T., Sugita, S., Genno, A., Takeuchi, K., Ozawa, T., Sakoda, Y., Nishimori, T., Takamura, K. and Akashi, H. 2001. Genomic and serological diversity of bovine viral diarrhea virus in Japan. *Arch. Virol.* **146**: 685–696. [Medline] [CrossRef]
20. Nagai, M., Hayashi, M., Itou, M., Fukutomi, T., Akashi, H., Kida, H. and Sakoda, Y. 2008. Identification of new genetic subtypes of bovine viral diarrhea virus genotype 1 isolated in Japan. *Virus Genes* **36**: 135–139. [Medline] [CrossRef]
21. National Livestock Breeding Center (NLBC). 2020. Statistical Survey on Livestock. https://www.id.nlbc.go.jp/data/toukei.html [accessed on March 3, 2021] (in Japanese).
22. National Veterinary Assay Laboratory (NVAL). 2018. Sales Amount of Medicines, Quasi-Drugs and Medical Devices for Animal Use. https://www.maff.go.jp/nval/iyakutou/hanbaidaka/index.html [accessed on September 17, 2020].
23. National Veterinary Assay Laboratory (NVAL). 2020. Annual Statistics of Animal Drug Approvals. https://www.maff.go.jp/nval/kentei_kensa/index.html [accessed on March 3, 2021] (in Japanese).
24. Newcomer, B. W., Walz, P. H., Givens, M. D. and Wilson, A. E. 2015. Efficacy of bovine viral diarrhea virus vaccination to prevent reproductive disease: a meta-analysis. *Theriogenology* **83**: 360–365.e1. [Medline] [CrossRef]
25. Omori, T., Inaba, Y., Morimoto, T., Tanaka, Y. and Kurogi, H. 1967. Bovine diarrhea virus. I. Isolation of non-cytopathogenic strains detectable by END method. *Jpn. J. Microbiol.* **11**: 133–142. [Medline] [CrossRef]
26. Presi, P. and Heim, D. 2010. BVD eradication in Switzerland-a new approach. *Vet. Microbiol.* **142**: 137–142. [Medline] [CrossRef]
27. Presi, P., Struchen, R., Knight-Jones, T., Scholl, S. and Heim, D. 2011. Bovine viral diarrhea (BVD) eradication in Switzerland--experiences of the first two years. *Prev. Vet. Med.* **99**: 112–121. [Medline] [CrossRef]
28. Saino, H., Kawauchi, K., Usui, A., Ohno, H., Sakoda, Y. and Tajima, M. 2013. Implementation and verification of the effectiveness of a regional control program for bovine viral diarrhea virus infection in Hokkaido, Japan. *Nippon Juishikai Zasshi* **66**: 791–796 (in Japanese).
29. Sakoda, Y., Ozawa, S., Damrongwatanapokin, S., Sato, M., Ishikawa, K. and Fukusho, A. 1999. Genetic heterogeneity of porcine and ruminant pestiviruses mainly isolated in Japan. *Vet. Microbiol.* **65**: 75–86. [Medline] [CrossRef]
30. Sekiguchi, S., Presi, P., Omori, R., Staerk, K., Schuppers, M., Isoda, N., Yoshikawa, Y., Umemura, T., Nakayama, H., Fujii, Y. and Sakoda, Y. 2018. Evaluation of bovine viral diarrhoea virus control strategies in dairy herds in Hokkaido, Japan, using stochastic modelling. *Transbound. Emerg. Dis.* **65**: e135–e144. [Medline] [CrossRef]
31. Simmonds, P., Becher, P., Bukh, J., Gould, E. A., Meyers, G., Monath, T., Muerhoff, S., Pletnev, A., Rico-Hesse, R., Smith, D. B., Stapleton J. T., ICTV Report Consortium. 2017. ICTV virus taxonomy profile: flaviviridae. *J. Gen. Virol.* **98**: 2–3. [Medline] [CrossRef]
32. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680. [Medline] [CrossRef]
33. Yeşilbağ, K., Alpay, G. and Becher, P. 2017. Variability and global distribution of subgenotypes of bovine viral diarrhea virus. *Viruses* **9**: 128. [Medline] [CrossRef]