FULL PAPER *Virology*

The Influence of Borna Disease Viral Infection on Dairy Cow Reproduction

Katsuro HAGIWARA[1)]*, Tatsuya ANDO[2)] and Masateru KOIWA[1)]

[1)]*School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069–8501, Japan*
[2)]*Ishikari Agricultural Mutual Relief Association, Hokkaido 067–0055, Japan*

(Received 27 July 2011/Accepted 11 November 2011/Published online in J-STAGE 25 November 2011)

ABSTRACT. We investigated the influence of Borna disease virus (BDV) infection on the clinical state of dairy cows. Sera from 149 cows were examined using enzyme-linked immunosorbent assay and western blotting detect antibodies to the BDV-nucleoprotein antigen. Among 149 investigated cows, 25 (16.8%) showed a positive reaction to BDV antigen. No significant difference existed in milk production or medical history between seropositive and seronegative cows. Although the estrus cycle appeared normal even in the seropositive cows, the frequency of artificial insemination and calving-to-conception intervals significantly increased in seropositive cows. Therefore, fertilization failure was recognized in the BDV-antibody positive cows.
KEY WORDS: borna disease virus, cow, sterility.

doi: 10.1292/jvms.11-0356; *J. Vet. Med. Sci.* 74(4): 419–421, 2012

Borna disease virus (BDV) is the causative agent of Borna Disease (BD), an immune-mediated neurological disease first described in horses, more than 200 years ago in southern Germany [11, 17, 18]. Initially, BD was thought to be restricted to horses and sheep in central Europe; subsequent epidemiological evidence has indicated that the prevalence and geographic distribution, as well as the host range of BDV, are broader than previously thought [16–18].

Epidemiological studies have documented the presence of serum antibodies to BDV and virus RNA in the brains of domestic animals and companion animals in several countries, including Japan [3, 5, 8, 14, 19]. Recently, BDV-infected dogs, cats, macaques, and raccoons have been reported in Japan [6, 10, 13, 15]. Dairy cows with BDV infection are recognized as seropositive individuals, although the infections are latent, and the cows never show any neurological symptoms. Conversely, studies regarding the prevalence of disease in BDV-antibody positive racehorses indicate that BDV infection may possibly contribute to an increase in the incidence rate of locomotion disorders in racehorses [9].

To investigate the prevalence of disease in BDV-antibody positive dairy cows on a farm, that have had Borna disease in the past, we conducted seroepidemiological studies and observed the clinical symptoms of 149 dairy cows in Hokkaido, Japan.

MATERIALS AND METHODS

Serological analysis: A total of 149 Holstein dairy cows (107 multiparous cows, 42 heifers) were examined for serological analysis to BDV antigen. The examined cows were born and reared at the same farm where a BDV-positive cow was confirmed in the past [14]. Serum samples were diluted at 1:100 with phosphate buffered saline containing 10% Block Ace (Dainippon Pharmaceutical Co., Osaka, Japan) and 0.05% Tween 20 and screened for antibodies to BDV by ELISA using the recombinant BDV nucleoprotein (BDV-N) antigen as described in our previous reports [4, 9]. To detect antigen-bound bovine immunoglobulin, a peroxidase-conjugated goat affinity purified anti-bovine IgG (Bethyl Laboratories, Inc., Montgomery, TX, U.S.A.) was used; positive reactions were identified using Microplate Imaging System (Ultramark, Bio-Rad, Hercules, Contra Costa, CA, U.S.A.) at 405 nm. The cutoff value for ELISA was calculated as the mean ± 2SD at OD of 405 nm of 5 intact cows (cutoff: OD, 0.4). ELISA-positive samples were further examined by western blotting using recombinant BDV-N as the target antigen. Antibody-antigen complexes were detected using the same peroxidase-conjugated goat affinity purified anti-bovine IgG mentioned above, as described elsewhere [5, 12].

Clinical records: The clinical records of cows were checked for 2 years before serological examination was conducted for quantity and quality of milk production, clinical disease history, and breeding history. The clinical records of the cows were investigated for the following conditions: dysfunction of locomotion (DL); dysfunction of the nervous system (DN), including cryptogenic behavioral changes with paralysis; and dysfunction of the alimentary systems (DA), including severe colic, penetration of the intestine, and peritonitis. The incidence and the factors related to the breeding conditions were determined on the basis of the following measures: lactation number, frequency of artificial insemination (AI), non-pregnant terms (the calving-to-conception intervals), and parturition season. Statistical analysis of disease incidence and seroprevalence were calculated using the chi-square test for independence.

*CORRESPONDENCE TO: HAGIWARA, K., School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069–8501, Japan.
e-mail: k-hagi@rakuno.ac.jp

Table 1. Summary of reproduction score and milk production data in seropositive cows

Multiparous cows Cow number	Non-pregnant terms (month)	AI	Number of births	Milk production	Milk Fat (%)	Milk Protein (%)
391	11.4	1	8	10900	4.2	3.0
416	14.0	3	7	9550	4.3	3.1
422	12.6	1	6	10885	4.9	3.3
458	14.3	4	5	10400	4.5	3.2
460	10.7	1	6	NA	NA	NA
481	14.6	1	5	8900	4.0	2.9
483	15.4	2	4	9700	4.3	3.3
501	24.2	9	2	12775	3.7	3.0
502	17.5	5	3	13200	3.8	3.0
503	12.6	2	3	9511	4.2	3.3
507	15.7	6	3	11000	4.2	3.0
543	15.0	4	2	12700	3.9	3.0
549	23.0	11	1	12637	4.2	3.2
551	24.6	10	1	11272	3.8	3.1
556	17.0	4	2	11700	3.4	3.0
558	20.9	9	1	12252	3.9	3.1
562	13.3	1	1	10028	4.5	3.3
567	11.6	1	1	12424	3.4	3.1
575	17.0	4	1	12300	3.6	2.9
576	14.1	3	1	10100	4.1	2.9
Heifers	First calving age	AI	Number of births	Milk production	Milk Fat	Milk Protein
629	29.9	5	0	–	–	–
668	27.3	2	0	–	–	–
669	29.7	5	0	–	–	–
702	26.8	4	0	–	–	–
703	23.2	1	0	–	–	–

AI: frequency of artificial insemination, NA: Data not available.

RESULTS

Our serological study indicated that 20 of 107 multiparous cows (18.7%) were diagnosed as seropositive cows. Among the heifers, 5 of 42 (11.9%) were seropositive. A total of 25 cows (16.8%) were seropositive for BDV. The average number of births was 3.4 in the seronegative group and 3.2 in the seropositive group. The clinical history showed that there was no significant difference in the clinical records (DL, DA, etc.) between the seropositive and seronegative cows. Clinical investigations showed the presence of neuroticism, hypodynamia, and dysstasia in some of the seropositive cows; however, this difference between the seropositive and seronegative cows was not statistically significant. No significant difference in the quantity and quality of milk production was observed between the groups. The average milk produced per milking period was 10,610 kg (seronegative) and 11,170 kg (seropositive). After investigation of the breeding condition, all the cows were found to be clinically normal; however, fertilization failure was observed in the seropositive cows. In multiparous cows, the frequency of AI increased significantly in the seropositive cows (average, 4.1) and prolonged non-pregnant terms (average, 16.0 months) as compared to the seronegative cows. Half of the antibody-positive cow enforced AI more than four times. The frequencies of AI and non-pregnant terms were 2.1 times and 13.5 months in seronegative cows, respectively (Tables 1 and 2, $P<0.05$). Interestingly, a similar phenomenon was observed in heifers. The frequency of AI increased significantly from 1.6 times (average of negative cows) to 3.4 times (average of positive cows). The first calving age was prolonged approximately 2 months, from 25.7 to 27.4 months (Table 2, $P<0.05$).

Table 2. Comparison of reproduction scores between seropositive and seronegative cows

Multiparous cows	Number of cows	NPT	AI	ELISA
Positive	20	16.0*	4.1*	0.59 ± 0.19
Negative	87	13.5	2.1	0.21 ± 0.04
Heifers	Number of cows	FC	AI	ELISA
Positive	5	27.4*	3.4*	0.65 ± 0.25
Negative	37	25.7	1.6	0.11 ± 0.01

AI: frequency of artificial insemination (average), *: $P<0.05$. NPT: average of non-pregnant terms (month) in multiparous cows. FC: average of first calving age in heifers, ELISA: the data are expressed as mean ± SD.

DISCUSSION

The seroprevalence of BDV in cows from the farm was 16.8%. The positive rate was higher in multiparous cows (18.7%) than in heifers (11.9%). The results were similar to a previous report in the Hokkaido region [8]. The frequency of AI in the seropositive cows increased to 4.1 times (average), implying a repeat bleeder. A repeat breeder was defined as a cow that did not become pregnant after 4 inseminations, despite no clinically detectable reproductive disorders [1]. The reproduction records of the seropositive cows showed no evidence of infection, leading to fertilization failure in the past 2 years. The estrus cycle appeared normal, even in the seropositive cows. There was no bias for the skill of AI practice among the cows, since one practitioner performed all AI procedures in this farm. The amount of feed was calculated by a program based on the milk production and body condition, and there was no deficiency in nutrients or quality.

Several studies have described the factors responsible for repeat breeding, such as environment, nutrition, and microorganisms [1, 2]. It is interesting that fertilization failure was also observed in seropositive heifers under nutrition management. All heifers were clinically normal, and their estrus cycles appeared normal. Infection by microorganisms is a cause of repeat breeding, but no particular microorganism that causes fertilization failure has been observed in this heard for the past 2 years.

In this study, we cannot elucidate the mechanism of fertilization failure in seropositive cows. However, an obvious reduction in breeding was observed in the seropositive cows. In our previous study, BDV RNA could be detected in a specific region of the brain from seropositive horses, but not in the uterus or ovaries from those animals [7]. BDV infection in the central nervous systems may be a factor that influenced the functions of female genital organs and hormone production. It is necessary to examine the influence that BDV infection in the central nervous system has on conception and the pregnancy.

ACKNOWLEDGMENTS. We thank T. Miura, D.V.M., and H. Iwao, D.V.M., for their technical assistance. We wish to express gratitude to the late Dr. Koichi Miyoshi, who helped conduct this research. This study was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Ayalon, N. 1978. A review of embryonic mortality in cattle. *J. Reprod. Fertil.* **54**: 483–493. [CrossRef] [Medline]
2. Azawi, O. I., Omran, S. N. and Hadad, J. J. 2008. A study of endometritis causing repeat breeding of cycling iraqi buffalo cows. *Reprod. Domest. Anim.* **43**: 735–743. [CrossRef] [Medline]
3. Bahmani, M. K., Nowrouzian, I., Nakaya, T., Nakamura, Y., Hagiwara, K., Takahashi, H., Rad, M. A. and Ikuta, K. 1996. Varied prevalence of Borna disease virus infection in Arabic, thoroughbred and their cross-bred horses in Iran. *Virus Res.* **45**: 1–13. [CrossRef] [Medline]
4. Hagiwara, K., Asakawa, M., Liao, L., Jiang, W., Yan, S., Chai, J., Oku, Y., Ikuta, K. and Ito, M. 2001. Seroprevalence of Borna disease virus in domestic animals in Xinjiang, China. *Vet. Microbiol.* **80**: 383–389. [CrossRef] [Medline]
5. Hagiwara, K., Kawamoto, S., Takahashi, H., Nakamura, Y., Nakaya, T., Hiramune, T., Ishihara, C. and Ikuta, K. 1997. High prevalence of Borna disease virus infection in healthy sheep in Japan. *Clin. Diagn. Lab. Immunol.* **4**: 339–344. [Medline]
6. Hagiwara, K., Matoba, Y. and Asakawa, M. 2009. Borna disease virus in Raccoons (*Procyon lotor*) in Japan. *J. Vet. Med. Sci.* **71**: 1009–1015. [CrossRef] [Medline]
7. Hagiwara, K., Momiyama, N., Taniyama, H., Nakaya, T., Tsunoda, N., Ishihara, C. and Ikuta, K. 1997. Demonstration of Borna disease virus (BDV) in specific regions of the brain from horses positive for serum antibodies to BDV but negative for BDV RNA in the blood and internal organs. *Med. Microbiol. Immunol. (Berl.)* **186**: 19–24. [CrossRef] [Medline]
8. Hagiwara, K., Nakaya, T., Nakamura, Y., Asahi, S., Takahashi, H., Ishihara, C. and Ikuta, K. 1996. Borna disease virus RNA in peripheral blood mononuclear cells obtained from healthy dairy cattle. *Med. Microbiol. Immunol. (Berl.)* **185**: 145–151. [CrossRef] [Medline]
9. Hagiwara, K., Okamoto, M., Kamitani, W., Takamura, S., Taniyama, H., Tsunoda, N., Tanaka, H., Iwai, H. and Ikuta, K. 2002. Nosological study of Borna disease virus infection in race horses. *Vet. Microbiol.* **84**: 367–374. [CrossRef] [Medline]
10. Hagiwara, K., Tsuge, Y., Asakawa, M., Kabaya, H., Okamoto, M., Miyasho, T., Taniyama, H., Ishihara, C., de la Torre, J. C. and Ikuta, K. 2008. Borna disease virus RNA detected in Japanese macaques (*Macaca fuscata*). *Primates* **49**: 57–64. [CrossRef] [Medline]
11. Ikuta, K., Ibrahim, M. S., Kobayashi, T. and Tomonaga, K. 2002. Borna disease virus and infection in humans. *Front. Biosci.* **7**: d470–d495. [CrossRef] [Medline]
12. Kishi, M., Nakaya, T., Nakamura, Y., Kakinuma, M., Takahashi, T. A., Sekiguchi, S., Uchikawa, M., Tadokoro, K., Ikeda, K. and Ikuta, K. 1995. Prevalence of Borna disease virus RNA in peripheral blood mononuclear cells from blood donors. *Med. Microbiol. Immunol. (Berl.)* **184**: 135–138. [CrossRef] [Medline]
13. Nishino, Y., Funaba, M., Fukushima, R., Mizutani, T., Kimura, T., Iizuka, R., Hirami, H. and Hara, M. 1999. Borna disease virus infection in domestic cats: evaluation by RNA and antibody detection. *J. Vet. Med. Sci.* **61**: 1167–1170. [CrossRef] [Medline]
14. Okamoto, M., Furuoka, H., Hagiwara, K., Kamitani, W., Kirisawa, R., Ikuta, K. and Taniyama, H. 2002. Borna disease in a heifer in Japan. *Vet. Rec.* **150**: 16–18. [CrossRef] [Medline]
15. Okamoto, M., Kagawa, Y., Kamitani, W., Hagiwara, K., Kirisawa, R., Iwai, H., Ikuta, K. and Taniyama, H. 2002. Borna disease in a dog in Japan. *J. Comp. Pathol.* **126**: 312–317. [CrossRef] [Medline]
16. Richt, J. A. and Rott, R. 2001. Borna disease virus: a mystery as an emerging zoonotic pathogen. *Vet. J.* **161**: 24–40. [CrossRef] [Medline]
17. Rott, R. and Becht, H. 1995. Natural and experimental Borna disease in animals. *Curr. Top. Microbiol. Immunol.* **190**: 17–30. [CrossRef] [Medline]
18. Staeheli, P., Sauder, C., Hausmann, J., Ehrensperger, F. and Schwemmle, M. 2000. Epidemiology of Borna disease virus. *J. Gen. Virol.* **81**: 2123–2135. [Medline]
19. Watanabe, Y., Yanai, H., Ohtaki, N., Ikuta, K. and Tomonaga, K. 2006. Prevalence of Borna disease virus antibodies in healthy Japanese black cattle in Kyushu. *J. Vet. Med. Sci.* **68**: 171–174. [CrossRef] [Medline]