

Borna Disease Virus Infection in Domestic Cats: Evaluation by RNA and Antibody Detection

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ABSTRACT. Borna disease virus (BDV) infection has been suggested to cause spontaneous neurological disease in cats referred to as staggering disease. However the evaluation of BDV infection in neurologically asymptomatic cats remained unclear. In the present study, BDV infected, asymptomatic cats in Tokyo were surveyed both by the presence of plasma antibodies against BDV-p24 and -p40 and by RNA detection in peripheral blood mononuclear cells. Seven of 32 domestic cats (21.9%) were serologically or genetically judged to be BDV-infected. Six cats were positive for anti-BDV antibody and two cats were positive for BDV RNA. Within the 2 RNA-positive cats, only one was positive for anti-BDV antibodies. Furthermore, the findings of anti-BDV-p40 and anti-BDV-p24 antibody-positive cats did not completely overlap. These results suggest that there are neurologically asymptomatic domestic cats infected with BDV present in the Tokyo area.—**KEY WORDS:** Borna disease virus, feline, infection.

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Borna disease virus (BDV) is an enveloped, nonsegmented, negative-stranded RNA virus [7] with at least six open reading frames; encoding nucleoprotein (p40), polymerase cofactor (p24), matrix protein (gp16), envelope protein (gp56), RNA polymerase (gp190) and x protein (p10) [5, 6, 30]. BDV causes neurological diseases characterized by behavioral disturbances and movement disorders in horses, sheep, cats, ostriches, rats and rabbits [26], but the precise mechanism underlying the diseases is poorly understood. BDV infection was originally identified in horses and sheep, but recent findings indicated that the virus naturally infects several vertebrate species [3, 4, 19, 21, 26, 31], and experimentally infects a broad range of animals [1, 12, 16, 25, 28, 29]. In cats, the spontaneous neurological disease referred to as staggering disease (SD), which is characterized by behavioral and motor disturbances [15], has revealed no relationship between neurological agents known to infect cats such as; feline leukemia or immunodeficiency virus, pseudorabies virus, canine distemper virus, *Borrelia burgdorferi* or *Toxoplasma gondii* [17]. Because a significant rate (44.0%) of cats suffering from SD were positive for anti-BDV antibody and cats experimentally infected with BDV showed SD, BDV has been implicated as one of the cause of SD [18–20]. It has also been reported that there were asymptomatic BDV infected cats [18, 24]. Nakamura *et al.* [24] suggested that there were two populations among BDV-infected cats (*i.e.*, individuals positive for BDV RNA in blood and individuals positive for anti-BDV antibody), however, unexpectedly, antibodies to BDV were not detected in individuals positive for BDV RNA in the blood.

In the present study, we evaluate BDV infection by testing antibody against BDV and BDV RNA in 32 randomly selected domestic cats hospitalized in animal hospital in the Tokyo area during 1996 and 1997. All cats had never

exhibited neurological disorders. Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-treated blood by centrifugation on Ficoll-Conray (density, 1.077–1.080 g/ml). Total RNAs derived from PBMCs were extracted using an RNA isolation kit (ISOGEN; Nippon Gene, Tokyo). BDV-p24 RNA was detected by nested reverse transcriptase-polymerase chain reaction (RT-PCR). Approximately one μ g of total RNA was reverse-transcribed and amplified by the first PCR using a commercial kit (Ez *rTth* RNA PCR kit; Perkin-Elmer, NJ) as previously described [13, 24]. Primer pairs used for reverse transcription and the first PCR were 5'-TGACCCAAC CAGTAGACCA-3' (nucleotides [nt] 1387 to 1405) and 5'-GTCCCATTCATCCGTTGTC-3' (nt 1865 to 1847). The second PCR was carried out using one μ l of the first PCR products as template and TaKaRa Taq DNA polymerase kit (TaKaRa Shuzo Corp.) [13, 24]. Primer pairs for the second PCR were 5'-TCAGACCCAGACCAGCGAA-3' (nt 1443 to 1461) and 5'-AGCTGGGGATAAATGCGCG-3' (nt 1834 to 1816). Reverse transcription and PCR were performed in a thermal cycler (GeneAmp PCR System 2400, Perkin-Elmer, NJ). PCR products were separated on a 2% agarose gel and visualized with ethidium bromide. The specificity of the amplification products was demonstrated by Southern blot hybridization with digoxigenin (DIG)-11-dUTP (Boehringer Mannheim, Germany) labeled oligonucleotides specific for BDV p24 nucleotides (5'-ATTGTCTCCATCATTTGTCTT CATGGAGCGATCTAGTATCT-3') (nt 1726 to 1687 of BDV strain V [27]) [22]. The hybridized probe was detected using a luminescent kit (DIG nucleic acid detection kit; Boehringer Mannheim, Germany). In order to determine the sensitivity of nested RT-PCR for BDV p24, total RNA of MDCK persistently infected with BDV (MDCK/BDV [9]) was 10-fold serially diluted with MDCK RNA and tested by nested RT-PCR. We found that as little as 100 fg

then were diluted 200-fold with PBS containing 0.05% Tween-20 for 1 hr at 4°C. The binding molecules were visualized with a commercial kit (ECL detection kit, Amersham, UK) using an enhancing chemiluminescence reagent. The cat plasma that reacted with GST-p24 or -p40 but not GST was judged to be serologically BDV positive.

As shown in Fig. 2, plasma from 6 cats (18.8%) reacted with BDV proteins examined using GST-p24 and -p40 as antigens. The total anti-BDV antibody-positive rate was higher than that previously reported by IFA in Germany (7.0%; [18]) and immunoblot analysis in Hokkaido (8.4%; [24]). Within the 6 cats, 5 (H1, H5, H14, H18 and H35) were positive for anti-BDV p24 and 5 cats (H1, H3, H5, H14 and H18) were positive for anti-BDV p40 antibodies. Also these cats, four (H1, H5, H14 and H18) were positive for both p24 and p40 antibodies (Fig. 2A). The anti-BDV p40 antibody-positive population did not necessarily overlap that of the anti-BDV-p24 antibody-positive population. To verify the specificity of reaction, positive plasmas were absorbed with purified GST-p24 and -p40; however no reaction was detected by immunoblot analysis (Fig. 2B). The summary of genetically and serologically surveyed cats is shown in Table 1. Within 32 cats, there were 26 healthy and 6 sick cats, respectively. Seven of 32 cats (21.9%) were genetically or serologically judged to be BDV-infected. Within the 2 RNA-positive cats, only 1 cat (H14) was positive for anti-BDV antibodies. The BDV-infected cats did not co-related by sex, age or clinical record. It might be easy to detect BDV infection in sick cats (4/6) compared with healthy cats (3/26).

In the present study, we observed BDV infection in neurologically asymptomatic cats at a significant rate: 7 of 32 cats (21.9%) were positive for anti-BDV antibody and/or BDV RNA. These results confirm previous report that neurological disease and encephalitis were not necessarily induced in cats experimentally infected with BDV and all cats which developed neurological signs recovered partly

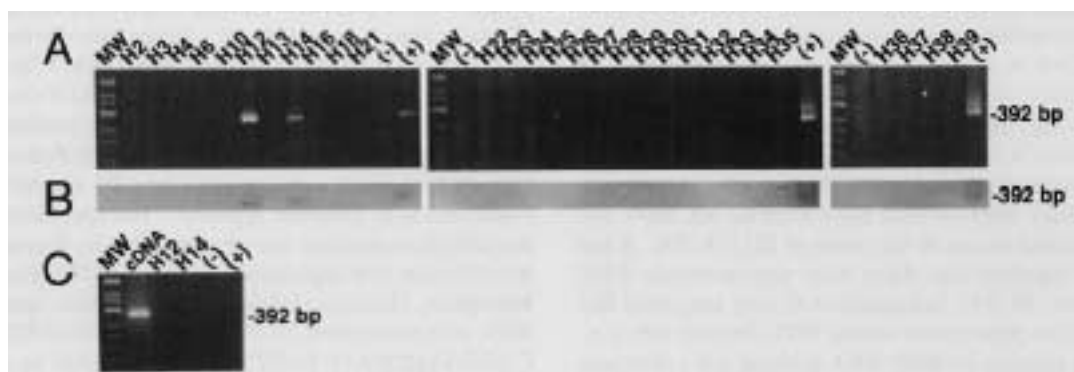


Fig. 1. The detection of BDV RNA in PBMCs from neurologically asymptomatic domestic cats during 1996 and 1997. (A) RNAs derived from PBMCs and cell lines were obtained by nested RT-PCR. The PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide. (B) The Southern blot hybridization of PCR products were conducted using a DIG-labeled BDV p24 oligomer as probe. The positive (+) and negative (-) reactions were conducted using RNA from MDCK cells infected with BDV persistently (MDCK/BDV), and uninfected MDCK cells, respectively. (C) RNAs derived from PBMCs (H12 and H14) and cell lines and cDNA of MDCK/BDV were obtained by nested PCR. Lane MW, size markers (ϕ 174/*Hinf* I digest).

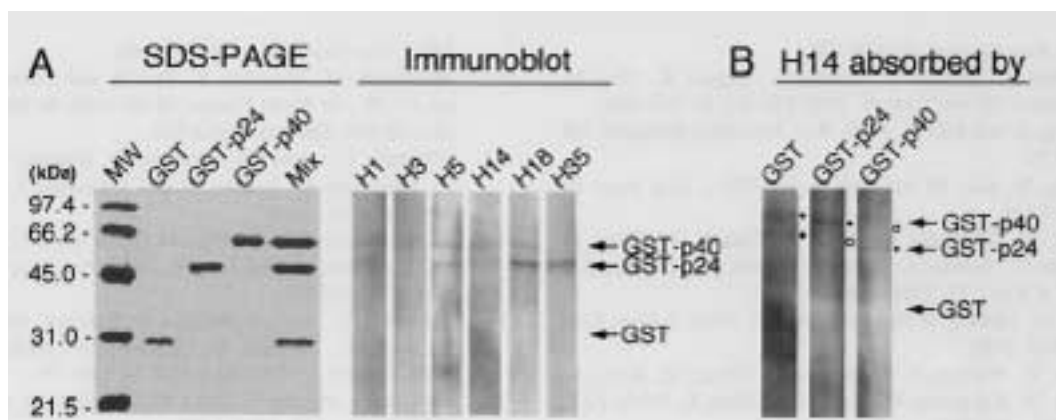


Fig. 2. Detection of anti-BDV antibodies in plasma from neurologically asymptomatic domestic cats during 1996 and 1997 by immunoblotting. (A) Equal moles of purified GST-fused BDV proteins (p24 and p40) and GST alone were electrophoresed simultaneously by SDS-polyacrylamide gel and transferred to PVDF membranes. Cat plasma absorbed with purified GST and diluted 200 times was incubated with the PVDF membrane, and the protein reactions were detected using peroxidase-conjugated anti-cat IgG antibody and the ECL reagent. (B) Reactivity of H14 plasma absorbed in GST, GST-p24 and GST-p40 respectively. Protein molecular weights were calculated by comparing their mobilities with those of marker proteins in a calibration kit (Bio-Rad).

Table 1. Profiles and summarized results of BDV infected neurologically asymptomatic cats

| Cat no. | Sex ^{a)} | Age | BDV RNA ^{b)} | Antibody to BDV ^{c)} -p24 | -p40 | Clinical record |
|---------|-------------------|---------|-----------------------|---------------------------------------|------|--|
| H1 | F | 9 yr | NT ^{d)} | + | + | Swelling of sublingual mucosa |
| H3 | M | 8 yr | — | — | + | Renal failure |
| H5 | M | Unknown | NT | + | + | Healthy |
| H12 | F | 7 mo | + | — | — | Healthy |
| H14 | M | 11 yr | + | + | + | Hepatitis |
| H18 | F | Unknown | — | + | + | Purulent dermatitis of lumbar region, Atrophic kidney |
| H35 | M | 1 yr | — | + | — | Healthy |

a) M, male; F, female. b) Results by nested RT-PCR of BDV-p24. c) Results by immunoblotting with BDV-p24 or -p40. d) Not tested.

from the acute stage of disease [20]. It has also been found that newborn rats, adult Syrian hamsters and mice experimentally infected with BDV survive also without overt neurological signs [1, 7, 10, 12, 25]. It is of interest to ask whether BDV has latently infected or already has been eliminated in these neurologically asymptomatic animals. We strongly suggest that evaluation of BDV infection in cats need to be surveyed genetically as well as serologically, using at least both BDV-p24 and -p40 antibodies or probes. More extensive epidemiological studies examining these criteria will be needed to establish relationships between persistent infection of BDV and some suspected neurological diseases, and the mechanisms of disease development on asymptomatic carriers of BDV.

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