NOTE   *Virology*

A Study on Borna Disease Virus Infection in Domestic Cats in Japan

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ABSTRACT. Borna disease virus (BDV) infection causes neurological disease in cats. Here, we report BDV infection in 199 hospitalized domestic cats in the Tokyo area. BDV infection was evaluated by detection of plasma antibodies against BDV-p24 or -p40. BDV-specific antibodies were detected in 54 cats (27.1%). Interestingly, the percentage of seropositive cats was not significantly different among the three clinical groups, i.e., healthy (29.8%), neurologically asymptomatic disease (22.2%) and neurological disease (33.3%). The specific antibodies were present even in cats aged below one year. The seropositive ratio was constant, irrespective of age and sampling season. The present study suggests that additional factors are required for onset of Borna disease in naturally infected cats and that BDV is transmitted through vertical routes in cats.
KEY WORDS: antibody, Borna disease virus, epidemiology, feline, vertical transmission

Borna disease virus (BDV), a nonsegmented, enveloped, negative-strand RNA virus of the *Bornaviridae* family [8], is the causative agent of Borna disease, an often fatal meningoencephalitis originally described in horses and sheep in Germany. Thereafter, natural BDV infection has been reported in many warm-blooded animals [15]. BDV persistently infects the central nervous system and often induces encephalitis [15]; experimental BDV infection has been shown to induce severe neurological disorders characterized by behavioral abnormality and movement disorders in laboratory rodents [15].

The domestic cat, *Felis catus*, is one of the natural hosts of BDV [8]. BDV causes staggering disease in cats, characterized by behavioral and motor disturbances with ataxia, behavioral change and loss of postural reaction [9–11]. Experimental BDV infection caused behavioral and motor disturbances with encephalitis in 4 cats among 7 infected

cats [11]. This indicates that persistent BDV infection is related to, but not sufficient for onset of staggering disease in cats. Consistent with these results, our previous study of 32 clinically healthy domestic cats living in the Tokyo area of Japan revealed more than 20% of the cats were infected with BDV [13]; however, the sample size of the previous study was relatively small. The present study further investigated BDV infection in healthy cats. In addition, BDV infection in cats with neurologically asymptomatic disease and those with neurological disease was also evaluated.

A total of 199 domestic cats living in the Tokyo area were examined for the present study. They were hospitalized at the Veterinary Teaching Hospital of Azabu University or four private animal hospitals between September 1996 and April 1999. The cats were categorized by veterinarians into 3 groups: clinically healthy (healthy: 94 cats), neurologically asymptomatic disease (NA: 81 cats) or neurological disease (ND: 24 cats). In addition, sampling date was categorized into 4 seasons on the basis of monthly average temperature in Tokyo as follows: spring − March, April and May (average temperature 8.5, 14.1 and 18.6°C, respectively); summer − June, July, August and September (21.7, 25.2, 27.1 and 23.2°C, respectively); fall − October and November (17.6 and 12.6°C, respectively); and winter − December, January and February (7.9, 5.2 and 5.6°C, respectively). Owners

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were briefed on the purpose of the study, and their consent was obtained. Blood samples (4 m*l*) collected in EDTA-coated tubes were immediately chilled at 4°C, and plasma was collected within 24 hr.

Expression and purification of glutathione-*S*-transferase (GST), GST-fused BDV p24 and GST-fused p40 were conducted as described previously [12]. Briefly, GST alone or GST-fused protein expressed in *E. coli* BL21 was purified near homogeneity by use of glutathione-Sepharose (GE Healthcare UK, Little Chalfont, Buckinghamshire, U.K.) and subsequent electroelution of recovered appropriate band after preparative SDS-PAGE. Detection of anti-BDV antibody was conducted by Western blot analyses. Nineteen pmol of GST, GST-BDV-p24 and GST-BDV-p40 were subjected to 12.5% SDS-PAGE and transferred to the PVDF membranes (Merch Millipore, Billerica, MA, U.S.A.). The membranes were blocked with skim milk (Block Ace, Dainippon Sumitomo Pharmaceuticals, Osaka, Japan) for 16 hr at 4°C. The membranes were then incubated with plasma, which was preadsorbed with GST and diluted with PBS containing 0.05% (v/v) Tween-20 (PBS-T) at 1:200 for 16 hr at 4°C. After washing with PBS-T for 40 min at 25°C, the membranes were reacted with peroxidase-conjugated anti-cat IgG antibody (Nordic-MUbio, Susteren, Netherlands) diluted at 1:6,000 with PBS-T for 40 min at 25°C. After washing with PBS-T for 40 min at 25°C, the immunoreactive molecules were visualized by use of ECL reagent (ECL detection kit, GE Healthcare UK).

When a significant band at GST-BDV p24 or p40 was detected, Western blot analyses were conducted again using the plasmas adsorbed with GST-BDV p24 or GST-BDV p40 beads, respectively. The cat plasma was judged to be seropositive if it reacted with GST-BDV proteins but not GST alone, and immunoreactions were reduced against GST-BDV proteins by adsorption with the respective protein beads (data not shown); cats with either anti-BDV p24 or p40 antibody were judged as those infected with BDV or BDV-related viruses. Infection with feline immunodeficiency virus (FIV) was also evaluated by the presence of anti-FIV antibody in plasma using a commercial kit (SNAP FeLV/FIV Combo, IDEXX Laboratories, Westbrook, ME, U.S.A.). Data were analyzed by χ^2 test to compare groups. Statistical significance was set at $P<0.05$.

BDV infection of domestic cats in the Tokyo area is shown in Table 1. A total of 54 cats among 199 cats (27.1%) were infected with BDV, and the percentage of BDV infection was not significantly different among the three groups: healthy (28/94, 29.8%), NA (18/81, 22.2%) and ND (8/24, 33.3%). Analyses on the relationship to clinical condition indicated a significantly higher BDV prevalence in cats suffering from stomatopathy (9/13, 69.2%) than in the healthy group ($P<0.05$) and in the ND group ($P<0.05$). Percentage of positive for anti-BDV p24 antibody was similar to that for anti-BDV p40 antibody in the healthy group and NA group. On the other hand, there tended to be more cats with anti-BDV p24 antibody than those with anti-BDV p40 antibody in the ND group.

Risk factors affecting BDV prevalence were next ana-

lyzed in domestic cats in the Tokyo area of Japan. Gender of cats did not affect BDV prevalence (data not shown). To examine the effect of age, cats were categorized into 4 groups: younger than one year (<1 yr), 1 to 4 years old (1–4 yr), 5 to 8 years old (5–8 yr) and 9 years old or older (>9 yr) (Table 2). Interestingly, the specific antibodies were present even in cats aged below one year (13/49, 26.5%), and the seroprevalence against BDV was relatively constant, irrespective of the age. The sampling season did not affect the seroprevalence against BDV, although it was slightly lower in fall than in the other seasons (Table 2).

The present study found that more than one-fourth of 199 cats in the Tokyo area of Japan are suspected to have been infected with BDV. Consistent with our previous study [13] and another study [4, 12, 14], a significant population of cats was infected with BDV without manifesting any clinical abnormalities. Even experimental BDV infection by intracranial injection did not cause Borna disease in 3 of 7 infected cats [11]. Therefore, it is thought that additional risk factor (s) is necessary for the onset of Borna disease in addition to BDV infection in naturally infected cats.

A previous study showed that higher BDV prevalence, which was judged by the presence of anti-BDV antibody, was detected in FIV-positive cats than in FIV-negative cats [3]; risk factors for FIV infection, such as fighting and biting, may overlap with those for BDV transmission in cats [3]. Multiple infections are possibly involved in the onset of neurological disorders in BDV-infected cats. Thus, we evaluated FIV infection. As a result, 2 cats among 8 BDV-infected cats were positive for FIV in the ND group, whereas no cat was positive for FIV among 18 BDV-infected cats in the NA group (data not shown). Clearly, further studies are needed because of the limited sample numbers, but FIV infection may trigger the induction of neurological diseases in BDV-infected cats [6].

A higher prevalence of BDV infection was detected in cats suffering from stomatopathy. Currently, the reason is unknown, and future experimental studies are needed to clarify the relationship between higher seropositive rate and stomatopathy; reactivation of persistent BDV infection as well as onset of stomatopathy may occur due to a reduction in the body's tolerance.

Currently, the transmission route of BDV is unclear in cats. There are 2 possibilities: horizontal transmission and vertical transmission. As for horizontal transmission of BDV in cats, reports have suggested that wild rodents transmit BDV to cats in Sweden and Finland [1, 7]. Insects are also candidates for transmitting BDV to cats. However, in Japan, BDV infection was not detected in wild rodents [16]. In addition, the present study found no effect of sampling season on BDV prevalence; if insects mediate BDV infection in cats, seroprevalence against BDV in cats would be affected by the sampling season because of the seasonal variation in the activity of insects. Thus, we think that horizontal transmission is not a major route in BDV infection in Japan. A previous study [14] that the age of cats had no effect on the seroprevalence against BDV supports this conclusion; the BDV seropositive population, which is persistently infected,

Table 1. Prevalence of anti-BDV antibodies in domestic cats in relation to their clinical condition

Clinical condition	Total subjects	Anti-BDV antibodies (%)[a]			
		Total	p24	p40	p24+p40
Healthy	94	28 (29.8)	20	22	14
Neurologically asymptomatic disease (NA)	81	18 (22.2)	14	13	9
LUTD[b]	15	4 (26.7)	4	3	3
Tumor[c]	18	1 (5.6)	1	0	0
Splanchnopathy[d]	15	6 (40.0)	5	3	2
Infectious disease[e]	14	3 (21.4)	2	2	1
Stomatopathy[f]	13	9 (69.2) *†	7	5	3
Trauma[g]	7	1 (14.3)	0	1	0
Others[h]	10	1 (10.0)	1	0	0
Neurological disease (ND)	24	8 (33.3)	7	3	2
Total	199	54 (27.1)	41	38	25

a) Figure within parenthesis indicates the percentage of positive to the total number within the same line. b) Lower urinary tract disease. Includes 2 cats with supervening infectious disease and 2 cats with supervening tumor or trauma. c) Includes 4 cats, 1 of each with supervening LUTD, stomatopathy, splanchnopathy or infectious disease. d) Includes 2 cats with supervening stomatopathy and 1 cat with supervening trauma. e) Viral infection and suppurative bacterial infection. Includes 4 cats, 1 of each with supervening LUTD, stomatopathy, splanchnopathy or trauma. f) Includes 3 cats with supervening splanchnopathy and 2 cats with supervening tumor or infectious disease. g) Including 2 cats, 1 of each with supervening LUTD or infectious disease. h) Dehydration, emaciation, depression, mixed connective tissue disease and dermatitis. *: $P<0.05$ as compared with the healthy group. †: $P<0.05$ as compared with the ND group.

Table 2. Effects of age and sampling season on BDV infection in cats

	Total Subjects[a]	Positive for BDV (%)[a]
Cat's age[b]		
<1 yr	49	13 (26.5)
1–4 yr	60	15 (25.0)
5–8 yr	31	10 (32.3)
>9 yr	36	10 (27.8)
Sampling season[c]		
Spring	53	17 (32.1)
Summer	41	12 (29.3)
Fall	33	5 (15.2)
Winter	70	19 (27.1)

a) Figure within parenthesis indicates the percentage of positive to the total number within the same line. b) Samples from 176 cats were analyzed. c) Samples from 197 cats were analyzed.

would increase with increasing age, if BDV was mainly transmitted through the horizontal route.

The findings of the present study on age-related BDV prevalence, i.e., more than one-fourth of cats aged below one year were infected with BDV, suggest the vertical transmission of BDV in cats in Japan. Vertical transmission has been reported also in horses [2]. In the present study, 12 cats among the 13 seropositive cats aged below one year were 6 to 11 month of age (data not shown). Considering that maternal antibodies disappear around 4 month of age in kittens [5], the presence of specific antibodies for BDV suggests antibody production in response to BDV infection by vertical transmission of BDV, but not maternal antibodies.

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