NOTE ***Virology***

Detection of Anti-Borna Disease Virus Antibodies from Cats in Asian Countries, Japan, Philippines and Indonesia Using Electrochemiluminescence Immunoassay

Yoichiro HORII[1], Joseph N. P. GARCIA[2], Deni NOVIANA[3], Fumi KONO[1], Takashi SAWADA[4], Toru NARAKI[4] and Kazunari YAMAGUCHI[5]

[1]Department of Veterinary Teaching Hospital and Internal Medicine, Faculty of Agriculture, Miyazaki University, Gakuen-kibanadai, Miyazaki 889–2192, Japan,[2]Veterinary Clinic, College of Veterinary Medicine, University of Philippines, Los Baños, Laguna 4031, Philippines,[3]Laboratory of Radiology, Faculty of Veterinary Science, Bogor Agricultural University, Bogor 16151, Indonesia, [4]Diagnostic Division, Eisai Tsukuba Research Laboratories, Tsukuba 300–2635 and [5]Blood Transfusion Service, Kumamoto University School of Medicine, Kumamoto 860–0816, Japan

(Received 28 November 2000/Accepted 16 April 2001)

ABSTRACT. Anti-Borna disease virus (BDV) antibodies were detected from cats in Japan, Philippines and Indonesia by using electrochemiluminescence immunoassay. Positive rates were 3.1%, 3.8% and 2.0% in Japan, Philippines and Indonesia, respectively. There was no differences in the positive rate of anti-BDV antibodies between male and female cats and among habitats. While, a significantly ($P<0.05$) higher prevalence (6.5%) was found in the oldest age group (more than 6 years) cats.

KEY WORDS: Borna disease virus (BDV), electrochemiluminescence immunoassay (ECLIA), feline.

J. Vet. Med. Sci. 63(8): 921–923, 2001

Borna disease is an infectious neurological disease that occurs sporadically in horses and sheep in Central Europe. Borna disease virus (BDV) is a noncytolytic, neurotropic, single strand, negative sense RNA virus that naturally infects a wide range of vertebrate species from birds and rodents to primates [1, 3]. BDV can also cause encephalomyelitis in a wide range of experimental animals [5, 6, 14].

In domestic cats, an characteristic disease of the central nervous system has been observed in Sweden [9]; the dominating clinical sign is an unsteady (staggering) gait. Histopathological examination of the cats showed that the inflammatory reaction of the central nervous system was most pronounced in the grey matter of the brain stem, basal ganglia and hippocampus. These changes and laboratory findings including leukopenia and elevations in protein content and white blood cell count of the cerebrospinal fluid in affected cats suggested that staggering disease is infectious and most probably caused by virus. By using an indirect immunofluorescence assay (IFA), anti-BDV antibody has been detected in the cats with typical staggering disease [10, 13]. Recently, the high prevalence of BDV infections has been demonstrated in domestic cats with neurological disorders in Japan [11].

A seroepidemiological survey of the cat has been done by using IFA and/or Western blot (WB) analysis also in Japan [12]. Since the antibody titers were usually very low in Borna disease, an establishment of sensitive and reliable method to detect a small amount of anti-BDV antibodies is required. Recently, we have developed a new system, electrochemiluminescence immunoassay (ECLIA), using recombinant BDV p24 and p40 proteins, which has the sensitivity and specificity to serve as a serological screening method for measuring anti-BDV antibodies [16]. In this study we tried to detect anti-BDV antibodies of domestic cats in Asian countries and to compare the prevalence of BDV among the countries.

Blood samples were collected from domestic cats at veterinary hospitals of University of Philippines Los Baños, Laguna, Philippines; Faculty of Veterinary Science, Bogor Agricultural University, Bogor, Indonesia; Osaka, Kitakyushu, Kumamoto and Nagasaki, Japan. Serum samples were stored at –30°C until use.

To measure antibody against BDV, ECLIA is performed as previously described [16]. Briefly, micro beads were coated with horse-derived BDV p24 and p40 recombinant proteins, then suspended in the bead buffer which contained 10% normal rabbit serum. The protein-coated beads were incubated with serum samples. Antibodies absorbed onto the micro beads were detected by electrochemically generated luminescence with the sandwich method using anti-cat IgG antibody coupled with Ruthenium (II) tris (bipyridyl); $Ru(bpy)_3^{2+}$ (IGEN International Inc., Gaithersburg, MD, U.S.A) [4]. The antibody level of each serum sample was expressed as a photocount (ECLIA count). In the first screening, a sample was considered temporarily positive if the ECLIA count was higher than the cutoff count (500) against mixed proteins (p24 + p40) based on the mean plus 5 standard deviations (SD) of the ECLIA counts from 13 cat samples which have been confirmed as seronegative for BDV by IFA. The temporarily positive sera were further examined with the following inhibition test to confirm the specificity. Each sample was preincubated with PBS containing 10% normal rabbit serum and recombinant p24 and p40 proteins at the concentration of 10 μg/m*l* for 15 min at 37°C. Serum sample preincubated with PBS containing 10% rabbit serum alone served as the control. After preincubation, each sample was measured by ECLIA as described [16]. A sample was declared anti-BDV antibody positive if the ECLIA count was inhibited to more than 50% of the control counts.

As shown in Table 1, 3.1%, 3.8% and 2.0% of cats in Japan, Philippines and Indonesia were anti-BDV antibody positive, respectively. Although the number of cats examined was not so huge, positive rates in respective countries were almost similar.

We made statistic analysis of the seropositive rate between sexes and between different habitats of the Japanese domestic cats. There was no differences in the positive rate of anti-BDV antibodies between males and females (2.4%; 4/164 and 3.4%; 6/176, respectively, Qi-square test, male vs. female; χ^2=0.28, NS). Also there was no significant difference in the seroprevalence of anti-BDV antibodies between the cats in Osaka and Nagasaki (3.4%; 2/59 and 3.6%; 10/275, respectively, Qi-square test, Osaka vs. Nagasaki; χ^2=0.009, NS). Of the 383 samples collected from Japan, 322 samples (host's age was known) were divided into 3 age groups based on their group size. Of the 107 samples from group I (0–1 year old), only one (0.9%) was positive for anti-BDV antibodies. In the group II (2–5 years old), 2 out of 108 samples (1.9%) were positive for anti-BDV antibodies. In the group III (more than 6 years old), 7 out of 107 samples were positive for anti-BDV antibodies. Significantly higher prevalence was found in the oldest group (group III, more than 6 years old) when compared to youngest group (group I, 0–1 year old) (Qi-square test, group I vs. group III; χ^2=4.675, P<0.05).

BDV antibody profiles in the seropositive cats and the conditions of each cat were summarized in Table 2. Anti-p24 antibody was more frequent than p40 antibody in almost all cases. In this experiment, we examined 5 cats which have neurological disorders, however, none of them showed seropositive against BDV (data not shown).

This study clearly shows that anti-BDV antibodies were detected from domestic cats in the Asian countries examined, viz. Philippines, Indonesia and Japan.

Nakamura *et al.* [11] reported that anti-BDV p24 and/or p40 antibodies were detected from 10 out of 15 cats with neurological disorders. In this experiment, we could not check a serum of the cat showing typical staggering disease, however, 5 serum samples from the cats with neurological disorders were checked for anti-BDV antibodies. Anti-BDV antibody was not detected from these cats, whereas the seropositive cases were detected regardless of host's clinical conditions and even from healthy cats. Regards to this, the profiles of BDV antibody of cat sera in this experiment is quite similar to the profiles of those in human healthy blood donors [16] both in which only p24 antibodies were noticeable. Borna disease has a variable period of incubation and diverse pathological manifestations depending on the species, immune status and age of the host, as well as route of infection and particular virus strain used for the infection. The course of infection varies from subclinical or slight behavioral abnormalities to a fatal neurological symptoms. Comparing to our results (3.4% positive in Osaka and 3.6% in Nagasaki), Nishino *et al.* [12] reported rather high prevalence (21.9%) of BDV infection also in neurologically asymptomatic cats in Tokyo, Japan. Although a big difference exists in the prevalence of BDV infection in cats between these studies, it is difficult to explain this discrepancy at present. However, there are some possibilities which may explain such a difference, e.g., differences in the research area and/or method of immunoassay. Regarding to

Table 1. Prevalence of anti-BDV antibodies of domestic cats in Japan, Philippines and Indonesia

Countries	Japan	Philippines	Indonesia
No. of samples examined by ECLIA	383	53	51
No. of positive	12	2	1
% positive	3.1	3.8	2.0

Table 2. Profiles of BDV antibody-positive Japanese cat serum samples

Cat number	Age (Year)	ECLIA count[a)] against p24+p40 (Inhibition %)	ECLIA count against p24	ECLIA count against p40	Clinical conditions
Group I					
1	1	2,261 (51.9)	4,109	37	abscess
Group II					
2	2	1,243 (58.5)	2,140	340	healthy
3	5	899 (81.6)	2,417	40	healthy
Group III					
4	6	894 (61.1)	1,207	180	healthy
5	8	1,212 (89.3)	2,368	101	trauma
6	8	6,514 (80.4)	10,464	662	renal failure
7	9	11,184 (92.4)	17,498	916	FIV infection[b)]
8	10	2,241 (87.0)	5,611	132	healthy
9	10	7,423 (75.9)	12,715	761	healthy
10	12	2,384 (87.5)	5,616	151	healthy

a) ECLIA count: a photocount of each sample measured by the automatic ECLIA analyzer.
b) FIV infection: feline immunodeficiency virus infection.

the ECLIA, it has been able to accurately identify experimentally infected rats and horses. In addition, the ECLIA corresponded to IFA in domestic horses that were also seropositive by a sensitive and specific Western blot assays [16].

Sporadic outbreaks of Borna disease have been reported in Germany [8]. There are several serological surveys from horses without giving rise to clinical signs in the Netherlands, and other European countries [8], the USA [7], Iran [2] and Japan [15], suggesting that the virus is more widely distributed than previously thought. In this paper, although we could not find any positive relationship between the detection of anti-BDV antibodies and the diseases such as neurological disorders in cats, we added Philippines and Indonesia as BDV-distributed countries. Further study is needed to clarify the significance and the role of anti-BDV antibodies in the BDV infection in animals.

ACKNOWLEDGEMENT. This work was supported in part by the Grant-in-Aid (2) 0904571 for scientific research B from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

1. Ashash, E., Malkinson, M., Meir, R., Perl, S. and Weisman, Y. 1996. *Avian Dis.* **40**: 240–245.
2. Bahmani, M. K., Nowrouzian, I., Nakaya, T., Nakamura, Y., Hagiwara, K., Takahashi, H., Rad, M. A. and Ikuta, K. 1996. *Virus Res.* **45**: 1–13.
3. Bignall, J. 1997. Staggering cats at Queens'. *Lancet* **349**: 1002.
4. Blackburn, G.F., Shah, H.P., Kenten, J.H., Leland, J., Kamin, R.A., Link, J., Peterman, J., Powell, M.J., Shah, A., Talley, D.B., Tyagi, S.K., Wilkins, E., Wu, T.G. and Massey, R.J. 1991. *Clin. Chem.* **37**: 1534–1539.
5. Briese, T., Schneemann, A., Lewis, A.J., Park, Y., Kim, S., Ludwig, H. and Lipkin, W.I. 1994. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 4362–4366.
6. Cubitt, B. and de la Torre, J.C. 1994. *J. Virol.* **68**: 1371–1381.
7. Kao, M., Hamir, A.N., Rupprecht, C.E., Fu, Z.F., Shankar, V. and Koprowski, H. 1993. *Vet. Rec.* **132**: 241–244.
8. Lange, H., Herzog, S., Herbst, W. and Schliesser, T. 1987. *Tierärztl. Umschau.* **12**: 938–946.
9. Lundgren, A.–L. 1992. *J. Comp. Pathol.* **107**: 411–425.
10. Lundgren, A.-L. and Ludwig, H. 1993. *Acta Vet. Scand.* **34**: 101–103.
11. Nakamura, Y., Watanabe, M., Kamitani, W., Taniyama, H., Nakaya, T., Nishimura, Y., Tsujimoto, H., Machida, S. and Ikuta, K. 1999. *Vet. Microbiol.* **70**: 153–169.
12. Nishino, Y., Funaba, M., Fukushima, R., Mizutani, T., Kimura, T., IIzuka, R., Hirami, H. and Hara M. 1999. *J. Vet. Med. Sci.* **61**: 1167–1170.
13. Nowotny, N. and Weissenbock, H. 1995. *J. Clin. Microbiol.* **33**: 1668–1669.
14. Richt, J.A., Vande Woude, S., Zink, M.C., Clements, J.E., Herzog, S., Stitz, L., Rott. R. and Narayan, O. 1992. *Clin. Infect. Dis.* **14**: 1240–1250.
15. Takahashi, H., Nakaya, T., Nakamura, Y., Asahi, S., Onishi, Y., Ikebuchi, K., Takahashi, T.A., Katoh, T., Sekiguchi, S., Takazawa, M., Tanaka, H. and Ikuta, K. 1997. *J. Med. Virol.* **52**: 330–335.
16. Yamaguchi, K., Sawada, T., Naraki, T., Igata-YI, R., Shiraki, H., Horii, Y., Ishii, T., Ikeda, K., Asou, N., Okabe, H., Mochizuki, M., Takahashi, K., Yamada, S., Kubo, K., Yashiki, S., Waltrip II, R.W. and Carbone, K.M. 1999. *Clin. Diag. Lab. Immunol.* **6**: 696–700.