NOTE *Clinical Pathology*

An Updated Nation-Wide Epidemiological Survey of Feline Immunodeficiency Virus (FIV) Infection in Japan

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ABSTRACT. An updated nation-wide epidemiological survey of feline immunodeficiency virus (FIV) infection was conducted in Japan. Blood samples were collected from 1,770 outdoor accessing cats from March to October 2008. Serologically, 410 cats (23.2%) were positive for anti-FIV antibody. Proviral DNA of the FIV *env* V3–V5 region isolated from 348 cases could be phylogenetically analyzed. The present study disclosed a geographic distribution of four subtypes (A, B, C and D) of FIV in Japan. Even though an FIV vaccine was introduced in Japan, we do not currently know whether this vaccine is effective against all strains of FIV in Japan or not. Therefore, close attention still has to be paid to epidemic and genotypic trends of FIV.

KEY WORDS: epidemiological survey, feline immunodeficiency virus, subtypes.

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Feline immunodeficiency virus (FIV) was first isolated from a cat with immunodeficiency-like symptoms in 1986, and is recognized as a causative agent for immunodeficiency and related immunological disorders in cats [1, 17]. To date, FIV is a widespread problem in feline species worldwide. Several epidemiological surveys of infection have been carried out since the discovery of FIV in Japan [8, 12]. The seropositivity for FIV infection in domestic cats in Japan was reported as high as 9.8% and 28.9% in the last two decades [8, 12]. However, the numbers of cats and areas evaluated were limited in these studies. It has been reported that FIV isolates can be divided into six subtypes, A to F, based on the molecular characteristics of the *env* or *gag* gene [2, 5, 16, 20, 21]. Previous studies revealed that four FIV subtypes including A, B, C and D were distributed in Japan [6, 9, 13, 15]. However, there is no updated information concerning the change in distribution pattern of each subtype in Japan. Therefore, a large-scale epidemiological survey would be essential to know the current situation in FIV infection prior to the distribution of an FIV vaccine. In this study, we have performed a nation-wide epidemiological survey of FIV infection in Japan.

Blood samples were collected from cats admitted to 47 private veterinary hospitals located in each of 47 prefectures in Japan from March to October 2008. Cats going outside of houses at least once a week were included; however, cats strictly kept indoors were excluded. Cats vaccinated with an FIV vaccine were also excluded from the analysis. Age, gender and chief complaints for each cat were recorded at each hospital. The status of FIV infection was initially screened by detection of anti-FIV antibody using a commercially available test kit (SNAP FeLV/FIV combo kit; IDEXX Laboratories Inc., Westbrook, ME, U.S.A.). The variables in clinical factors associated with the presence of anti-FIV antibody were assessed with crude odds ratios (ORs) calculated by a χ^2-test within 95% confidence intervals (CIs). Nested PCRs were carried out for detection of *env* (V3–V5) and *gag* regions of FIV proviral DNA in the peripheral blood [6, 15]. The nucleotide sequences of FIV *env*-derived proviral DNA were determined by direct sequencing, and phylogenetic analysis was performed by a neighbor-joining method in the DNADIST program from the PHYLIP software package to estimate subtypes of FIV as previously reported [3, 15].

The final number of cats included in this study was 1,770, with their profiles shown in Table 1 and Fig. 1. Of the examined cats 1,695 were mixed breed, other breeds included 26 American short hair, 5 Persian, 4 Abyssinian and 40 others. The gender of cats in this cohort consisted of 939 male, 825 female and 6 unknown. Records of gender in six cases were not available because of lack of data at veterinary hospitals. The age of these cats varied ranging from juvenile to adult; however, many of the cats were 0.5–10 years old (Fig. 1). Of the 1,770 cats, 1,175 cats were admitted to the veterinary hospital due to clinical signs including injury, anorexia, emaciation, eye and nasal discharges, anemia, chronic kidney diseases, dyspnea, and difficulty of micturition. Remaining cats were referred for health check and/or vaccination. A history of bite wound suffered in fighting was recorded in 716 cases based on the declaration of cat owners.

As shown in Table 1, 23.2% of the 1,770 cats were seropositive for FIV. A trend for seropositivity in breeds could be detected in mixed breed cats against pure breed cats

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Table 1. Profile and FIV sero-positivity of examined 1,770 cats

	Number of cats	(%)	Cats with anti-FIV Ab	(%)	Odds ratio
Total	1,770	(100%)	410/1,770	(23.2%)	
Breed					
Mixed breed	1,695	(95.7%)	403	(22.8%)	3.0*
American short hair	26	(1.5%)	4	(0.2%)	
Persian	5	(0.3%)	1	(0.1%)	
Abyssinian	4	(0.2%)	0	(0.0%)	
Other breeds	40	(2.3%)	2	(0.1%)	
Gender					
Male	939	(53.1%)	292	(16.5%)	2.7
Female	825	(46.6%)	117	(6.6%)	
Unknown	6	(0.3%)	1	(0.1%)	
History of bite wound suffered in fighting					
Yes	716	(40.5%)	250	(14.1%)	3.0
No	956	(54.0%)	142	(8.1%)	
Unknown	98	(5.5%)	18	(1.0%)	
Some sort of clinical signs					
Yes	1,175	(66.4%)	332	(18.8%)	2.7
No	586	(33.1%)	74	(4.2%)	
Unknown	9	(0.5%)	4	(0.2%)	
Anamnestic history					
Yes	761	(43.0%)	219	(12.4%)	1.7
No	935	(52.8%)	176	(9.9%)	
Unknown	74	(4.2%)	15	(0.9%)	
Stomatitis/Gingivitis					
Yes	488	(27.6%)	180	(10.2%)	2.8
No	1,216	(68.7%)	210	(11.9%)	
Unknown	66	(3.7%)	20	(1.1%)	

* Mixed breed cats vs pure breed cats.

(OR=3.0, CI=95%; Table 1). All kittens less than six months old were seronegative, however juvenile cats (0.5–2 years old) revealed 3.4–14.4% prevalence (Fig. 1). Adult cats (≥ 2 years old) showed a high infection rate around 28–30%. The presence of anti-FIV antibody was observed in 292 (16.5%) male and 117 (6.6%) female cats. Male cats constituted 71.2% of all seropositive animals and exhibited a greater risk of infection than females (OR=2.7, CI=95%; Table 1). The seropositivity in 716 cats with a history of fighting wounds was 14.1%, significantly higher (OR=3.0, CI=95%) than the 8.1% in cats without that history. Seroprevalence in cats with and without any clinical signs was 18.8 and 4.2%, respectively. Cats infected with FIV were highly likely to exhibit clinical signs (OR=2.7, CI=95%). Cats with anamnestic history (OR=1.7, CI=95%) and/or oral lesions (OR=2.8, CI=95%) exhibited higher infection rates than cats without those factors.

Proviral DNA was detected in 404 of the 410 seropositive cats by *env*, *gag* or both PCR analyses. Nineteen seronegative cats were positive by PCR analysis. The concordance of results between the antibody test and *env* PCR was 96.7% (both positive, 370 cases; antibody positive/PCR negative, 40 cases; antibody negative/PCR positive, 19 cases; both negative, 1,341 cases). The *gag* PCR was performed in 40 cases which showed discordant results between serological test and *env* PCR (antibody positive/PCR negative). The combined results of *env* and *gag* PCRs led an increased concordance to 98.6% (both positive, 404 cases; antibody positive/PCR negative, 6 cases; antibody negative/PCR positive, 19 cases; both negative, 1,341 cases). These different concordances might have been due to the different sensitivity between *env* and *gag* PCR systems, or a possible emergence of FIV strain with a significant change in *env* gene which cannot be detected in our *env* PCR system.

Nucleotide sequences of the FIV proviral *env* V3–V5 region from 348 cases was determined and phylogenetically analyzed. The phylogenetic analysis revealed that four subtypes (A, B, C and D) of FIV were distributed throughout Japan (Fig. 2A). The most common FIV subtype was B (42.2%) followed by A (30.2%), D (22.1%) and C (5.5%). Each prefecture in Japan was assigned a three letter code, and geographical locations and regional distribution of FIV were shown in Fig. 2B. Most of subtype A FIVs was observed in west part of Japan and Hokkaido. In contrast, subtype B viruses mainly distributed in east part of Japan and urban areas. Subtype D viruses were found in Kinki and

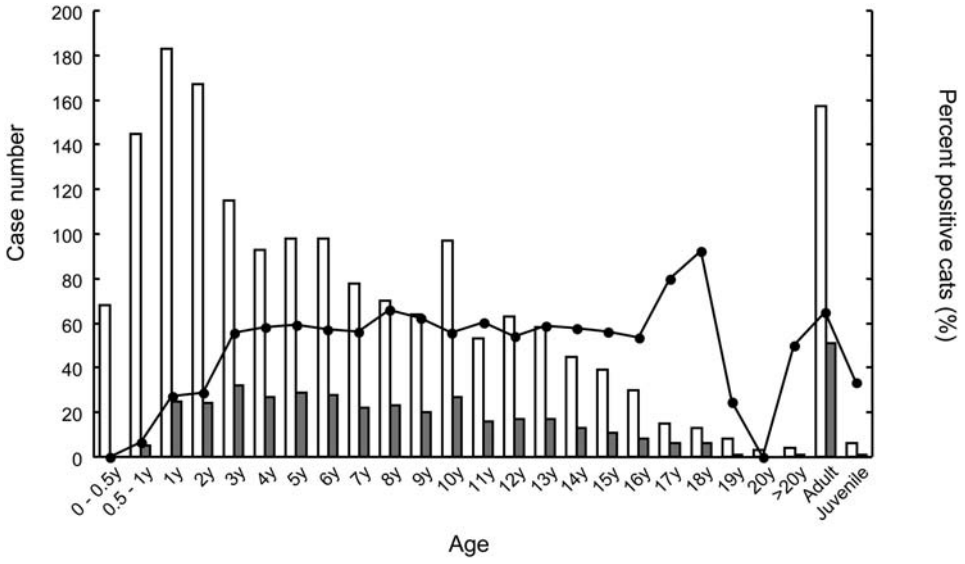

Fig. 1. Case numbers and seropositive cases in each categorized age. White and grey bars show the numbers of examined cases and seropositive cases, respectively. Circles represent the percent seropositive cats in each age range.

northern Kyushu areas. Subtype D viruses could possibly be divided into two further subtypes: D1 and D2. The possible emergence of D2 virus has been shown in a previous report as Old D viruses [13]. Our study also clarified this type of virus was mainly distributed in Hokuriku area (Fig. 2B). Limited information about subtype C in Japan has been available [13, 15]; however a number of subtype C FIV-infected cases were detected especially in Chubu area in this study.

To our knowledge, this is the first report on the nationwide survey of FIV infection in Japan. The FIV seropositivity of cats in this study was still found to be higher than those in previous reports in Japan and other countries [4, 7, 11, 12, 20, 24]. The cat population that went outside at least once per week was used in this study, conceivably resulting in the high seropositivity; however, opportunity of exposure to FIV might be very frequent in Japan.

Major objective of this study was to reveal the updated distribution pattern of FIV subtypes. As previously described, we also found that there were five subtypes of FIV in Japan and that distribution pattern of major 3 subtypes, A, B and D (D1), was similar [9, 13, 15]. Major difference of our results from previous ones is a higher proportion of subtype C viruses. Although this subtype had previously been detected in only Aichi (AIC) prefecture, subtype C FIV was mainly found in the present study in Mie (MIE) prefecture where an epidemiological survey has not been performed since the discovery of FIV-infected cats in Japan [9, 15]. Therefore, conceivably subtype C FIVs existed dominantly in this area and would possibly spread to surrounding areas including AIC, GIF, SIG, KYT and HYG prefectures. As previously reported by Mochizuki *et al.*, we also found the existence of D2 viruses represented by strain VND-1 [13, 14]. Its distribution was relatively limited to Hokuriku area. The origin of this subtype of virus might have been from D1 FIV in this area or abroad; however, we cannot conclude it.

Blood samples were collected in a rush just prior to the market introduction of a vaccine. This vaccine was reported to prevent the infection of subtypes A, B and D FIV but we have no information of its efficacy against subtype C [10, 18, 19, 22, 23]. Previous studies indicated the presence of subtype C FIV in Japan; however, it has been considered that subtype C virus is rare in Japan [13, 15]. Surprisingly, we found that subtype C FIV was the major subtype in Chubu and Kinki areas. An FIV vaccine has been introduced into the Japanese market at the time of writing; however verification of its efficacy against subtype C FIVs will be required as soon as possible. Similar concern could also be applied to subtype D2 FIVs. VND-1 strain and others were obviously derived from a main branch of subtype D viruses. These viruses may also have different antigenicity in the envelope protein compared with vaccine strain, Shizuoka (D1). Although we have not evaluated the subtypes of *gag* gene, further information would be available concerning detailed viral genotyping and frequency of recombination if it is conducted.

In this study, we carried out a nation-wide epidemiological survey of FIV in cats. Despite introduction of an FIV vaccine in Japan, we do not currently know whether this vaccine is effective against all strains of FIV in Japan or not. Therefore, close attention still has to be paid to epidemic and genotypic trends of FIV.

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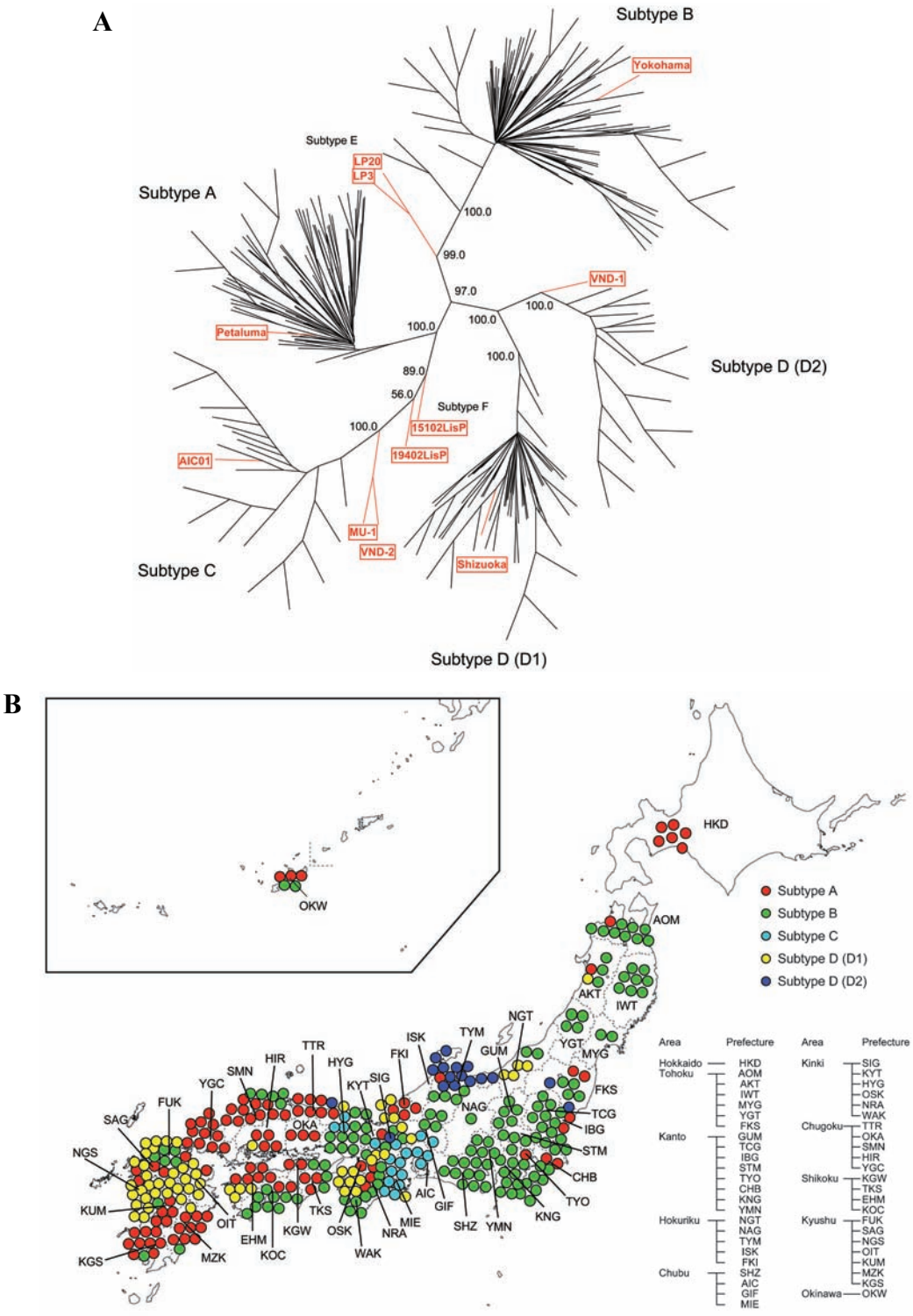

Fig. 2. Phylogenetic analysis and distribution of FIV subtypes. (A) An unrooted neighbor-joining phylogenetic tree of the FIV *env* gene covering variable regions V3–V5. The eleven FIV clones previously reported were used as subtype markers and are shown in red characters: Subtype A, Petaluma (GenBank/EMBL/DDBJ accession No. M25381); Subtype B, Yokohama (D37812); Subtype C, AIC01 (AB010396), MU-1 (AB016666) and VND-2 (AB083503); Subtype D1, Shizuoka (D37811); Subtype D2, VND-1 (AB083502); Subtype E, LP3 (D84496) and LP20 (D84498); Subtype F, 15102LisP (DQ072567) and 19402LisP (DQ072572). The numbers at each branch point indicate the bootstrap values preserved through greater than 50 in 100 bootstrap repetitions. Nucleotide sequences of the FIV genome obtained in this study have been deposited in the DDBJ database under accession numbers AB514955 through to AB51530 and their predicted subtypes are listed in the appendix. (B) Geographic distribution of each subtype of FIV detected in 348 cases. Each dot represents one FIV-infected cat. A three-letter code assigned to each prefecture. These 47 prefectures were divided into ten areas.

Science.

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APPENDIX

The 348 FIV *env* clones obtained in this study were categorized as follows: **Subtype A**, AKT18, AOM38, CHB3, CHB6, EHM2, EHM12, EHM13, EHM14, EHM22, EHM31, FKI3, FKI5, FKI13, FKI37, FKS31, FKS39, FUK24, HIR5, HIR18, HIR21, HIR28, HKD3, HKD7, HKD15, HKD18, HKD26, IBG14, IBG32, KGS1, KGS3, KGS6, KGS15, KGS17, KGS18, KGS21, KGS27, KGS28, KGW16, KGW20, KOC8, KOC29, KUM5, KUM12, KUM19, KUM25, KUM40, MZK11, MZK15, MZK20, MZK23, MZK27, MZK35, MZK36, MZK37, MZK40, NGS6, NGS17, NRA33, NRA35, NRA38, OIT9, OKA11, OKA14, OKA28, OKW5, OKW24, OKW27, OSK24, SAG11, SAG18, SIG21, SMN5, SMN6, SMN10, SMN11, SMN12, SMN13, SMN14, SMN19, OKA11, OKA14,

OKA28, OKW5, OKW24, OKW27, OSK24, SAG11, SAG18, SIG21, SMN5, SMN6, SMN10, SMN11, SMN12, SMN13, SMN14, SMN19, SMN20, SMN26, SMN32, TKO19, TKS28, TKS31, TKS39, TTR1, TTR4, TTR10, TTR11, TTR14, TTR15, TTR28, TYM1, YGC5, YGC12, YGC13, YGC14, YGC18, YGC23, YGC24, YGC29, YGC30, YGC33, and YGC37; **Subtype B**, AKT1, AKT7, AKT28, AOM1, AOM7, AOM9, AOM10, AOM20, AOM23, AOM26, AOM33, AOM34, CHB1, CHB8, CHB12, CHB16, CHB17, CHB23, CHB25, CHB33, FKS6, FKS7, FKS11, FKS32, FUK19, FUK20, FUK23, FUK33, GIF6, GIF7, GIF10, GIF30, GUM2, GUM6, GUM10, GUM16, GUM35, HYG6, HYG14, HYG18, HYG23, HYG32, HYG36, IBG2, IBG3, IBG18, IBG28, IBG34, IBG35, IBG39, IWT2, IWT10, IWT19, IWT27, IWT29, IWT30, IWT34, IWT39, KGS20, KGW1, KGW25, KNG4, KNG14, KNG16, KNG17, KNG18, KNG24, KNG25, KNG27, KNG31, KOC2, KOC4, KOC17, KOC23, KOC26, KOC27, KOC37, KOC38, KOC39, KUM2, KYT7, KYT8, KYT10, KYT26, KYT29, MYG7, MYG25, MZK30, NAG3, NGT4, NGT38, NRA6, NRA8, NRA12, NRA27, NRA31, OKW4, OKW33, OSK2, SAG33, SHZ5, SHZ8, SHZ16, SHZ18, SHZ19, SHZ20, SHZ21, SHZ22, SHZ23, SHZ31, SHZ32, SIG32, SIG34, SIG35, SMN4, SMN7, SMN16, SMN22, SMN24, STM13, STM2, STM4, TCG2, TCG3, TCG6, TCG13, TCG35, TKS20, TKS32, TYM2, TYM5, TYM13, TYM29, TYO16, WAK1, WAK4, WAK29, WAK40, YGT2, YGT3, YGT9, YGT22, YMN13, YMN18, YMN25, YMN37, and YMN40; **Subtype C**, AIC7, AIC8, AIC13, GIF23, GIF29, HYG4, HYG22, HYG34, KYT37, MIE1, MIE2, MIE3, MIE11, MIE15, MIE16, MIE24, MIE25, MIE32, and SIG26; **Subtype D1**, AKT22, EHM9, EHM18, EHM20, EHM28, FKI28, FKI7, FUK25, FUK26, FUK29, FUK37, FUK5, HIR12, HYG24, KUM4, KUM6, KUM11, KUM16, KUM22, KUM24, KUM28, KUM33, KUM35, KUM37, KUM39, MIE40, MZK38, NGS12, NGS18, NGS22, NGS23, NGS30, NGS37, NGS40, NGT12, NGT16, NGT19, NGT37, NRA2, NRA19, NRA20, OIT1, OIT16, OIT18, OIT23, OIT37, SAG12, SAG14, SAG16, SAG28, SAG40, SIG2, SIG5, WAK3, WAK13, WAK14, WAK16, WAK24, WAK25, and WAK39; **Subtype D2**, FKS14, IBG1, ISK5, ISK11, ISK14, ISK21, NGT9, NGT31, SIG30, TTR30, TYM8, TYM14, TYM28, TYM30, TYM31, TYM32, and TYM40.