

## Recent Epidemiological Status of Canine Viral Enteric Infections and *Giardia* Infection in Japan

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**ABSTRACT.** Epidemiology of canine enteric infections was studied. Rectal swabs collected from 95 dogs presented at animal hospitals during a period from January to June of 2000 were examined for enteric pathogens, including viruses and *Giardia lamblia* (*G. lamblia*). Most frequently detected in both diarrheal and normal feces were canine coronavirus (55.4%) and *G. lamblia* (48.2%). Canine parvovirus type 2 (CPV-2) was specifically associated with diarrheal cases and CPV-2b was the predominant antigenic type. Although canine rotavirus, canine adenovirus, and canine distemper virus were also detected in a small number of diarrheal cases, no evidence for calicivirus infection was obtained.

**KEY WORDS:** canine coronavirus, canine parvovirus, *Giardia lamblia*.

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Type 2 canine parvovirus (CPV-2), canine distemper virus (CDV) and canine adenovirus type 1 (CAV-1) have been well established as primary enteric viral pathogens of dogs. Secondly, canine coronavirus (CCV) as well as canine rotavirus (CRV) have been more recently documented [12]. In this communication, we describe etiology of current canine diarrheal cases in Japan in consideration of more efficacious preventive measures matching the existing epidemiological status.

A total of 95 rectal swab specimens were taken from dogs, and the samples consisted of 85 diarrheal and 10 normal stools. These samples were submitted by animal hospitals in various locations of the country (Hokkaido, Miyagi, Fukushima, Ibaraki, Tokyo, Kanagawa, Shizuoka, Osaka, Okayama, and Fukuoka) during a period from January to June of 2000. Ages of dogs were between 28 days and 4 years, and the average was 4.1 months old. Two swab specimens were taken from each patient, and one was used for virus examination and the other for *Giardia lamblia* (*G. lamblia*) detection by enzyme-linked immunosorbent assay (ELISA). Stool was also examined for *Giardia* cysts by a zinc sulfate centrifugation flotation method at each animal hospital.

Swabs were placed in 2 ml of Eagle's minimal essential medium, extracts were clarified by centrifugation at 15,000 rpm for 20 min, and the resulting supernatants were examined for viruses.

For detection of CRV, reverse passive hemagglutination assay (RPHA) and virus isolation by using MA104 cell culture were used, and the methods have been described previously [15]. Two diarrheal specimens were found to be CRV antigen-positive by RPHA as shown in Table 1. Antigenic type G3P [3] rotavirus strain KSD-88 belonging to the K9 genogroup, determined by sequencing, PCR, and RNA-RNA hybridization assays described elsewhere [17, 18], was isolated from one specimen, but not from the other

because of interference by bacterial contamination in the cell culture. The detection rate of CRV in the present study was low as that of the previous survey [12] and the antigenic type was the same as the previous rotavirus isolates reported [18].

For detection of CCV, reverse transcriptase-PCR (RT-PCR) assay and virus isolation by using fcfw-4 cell culture were used, and the methods have been described previously [5]. Forty-seven out of 82 diarrheal specimens (57.3%) were found to be CCV RNA-positive using the amplicon with a specific cutting site of restriction enzyme *Dra* I (data not shown), as described previously [8]. In the normal feces CCV RNA was also detected in four out of 10 specimens. However an intact CCV was isolated from only two diarrheal specimens and this was almost the same virus-isolation rate as the previous report [5], suggesting a fastidious nature of CCV *in vitro*.

For detection of CPV-2, viral DNA was examined by PCR assay described previously [19] and found in 21 (25%) out of 84 diarrheal specimens. For virus isolation, both CRFK and MDCK cell cultures were used for each specimen as described previously [14] and 21 isolates were obtained from 90 specimens tested. One normal feces was also found to be DNA-positive but virus was not recovered from the sample. There was no obvious difference in sensitivity of each cell type against recent field CPV-2 at the point of isolation rate, but it appeared that virus grew more efficiently in the MDCK than CRFK cell cultures (data not shown). Antigenic typing of 18 isolates by using hemagglutination inhibition (HI) test with a panel of monoclonal antibodies was performed. The method of HI test was described previously [16] and the monoclonal antibodies (A3B10, A4E3, C1D1, B4A2, and B4E1) were obtained from James A. Baker Institute for Animal Health of Cornell University. Sixteen isolates were typed as 2b and the remaining two were typed as 2 and 2a, respectively (data not shown). Both

Table 1. Detection of enteric viruses and *Giardia lamblia* (*G. lamblia*) from canine fecal specimens obtained during a period from January to June, 2000

Condition of feces	CRV <sup>a)</sup>		CCV		CPV-2	
	RPHA <sup>b)</sup>	VI <sup>c)</sup>	RT-PCR	VI	PCR	VI
Diarrheal	2/85 (2.4%)	1/85 (1.2%)	47/82 (57.3%)	2/79 (2.5%)	21/84 (25%)	21/81 (25.9%)
Normal	0/10	nt <sup>d)</sup>	4/10 (40%)	0/9	1/10 (10%)	0/9
Total	2/95 (2.1%)	1/85 (1.2%)	51/92 (55.4%)	2/88 (2.3%)	22/94 (23.4%)	21/90 (23.3%)

  

Condition of feces	CAV	CDV	Calicivirus		<i>G. lamblia</i>	
	VI	RT-PCR	RT-PCR	VI	ZSCF	ELISA
Diarrheal	2/81 (2.5%)	8/84 (9.5%)	0/71	0/67	14/68 (20.6%)	35/71 (49.3%)
Normal	0/9	2/10 (20%)	0/10	0/9	4/9 (44.4%)	4/10 (40%)
Total	2/90 (2.2%)	10/94 (10.6%)	0/81	0/76	18/77 (23.4%)	39/81 (48.2%)

a) CRV, canine rotavirus; CCV, canine coronavirus; CPV-2, canine parvovirus type 2; CAV, canine adenovirus; CDV, canine distemper virus.

b) RPHA, reverse passive hemagglutination; VI, virus isolation in cell culture; RT-PCR, reverse transcriptase PCR; ZSCF, zinc sulfate centrifugal flotation; ELISA, enzyme-linked immunosorbent assay by ProSpecT<sup>®</sup> (Alexon-Trend, Inc., MN)

c) MA104, fcfw-4, and MDCK cell cultures were used for isolation of CRV, CCV, and CAV, respectively. Both MDCK and CRFK cell cultures were used for isolation of CPV-2 and calicivirus.

d) nt, not tested.

CRV and CCV were detected in the stool from which the antigenic type 2 CPV was recovered.

Two CAV isolates were recovered from 81 diarrheal feces and their serotype specificity was molecularly determined by PCR assay. For identification of serotype 1 a primer set of CAVE31 (5'-GCGGATCCCGTTCATCTTT CAGCCC-3') and CAVE32 (5'-GCGGTACCAGCCAT-AGTCCCATGGACCAG-3') was selected in the E3 region of CAV genome. It was made based on the sequence information of Glaxo strain and an expected PCR product was 998 bp. For serotype 2 a primer set of CAVEf (5'-CCCT-GCGTCATCACCCAAAGTAGC-3') and CAVE34 (5'-TAGGGCCCCATTAGAAGGCTGAGGGTGG-3') was made based on the sequence information of Toronto A26/61 strain and an expected PCR product was 2,906 bp. Accession numbers of Glaxo and Toronto A26/61 strains in the DDBJ/EMBL/GenBank databases were M60937 and U77082, respectively. Amplification was performed with 20 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 45 sec, and extension at 72°C for 5 min. Both isolates were found to belong to serotype 2 CAV.

For detection of CDV, RT-PCR assay, which detects a portion of viral hemagglutinin gene, was used according to the method described previously [10, 13], and found that 8 diarrheal and two normal fecal specimens contained CDV.

Detection of calicivirus was performed by both virus isolation, in canine MDCK and feline CRFK cell cultures, and by RT-PCR assay [7], but no positive proof was obtained.

A flagellate binucleate protozoan parasite *Giardia* was examined by a commercially available ELISA test ProSpecT<sup>®</sup> *Giardia* Microplate assay (Alexon-Trend, Inc., MN). The test detects a 65,000-dalton glycoprotein that is produced by *G. lamblia* as they multiply within the host

intestinal tract, and this is a *Giardia* specific antigen showing no cross reactivity with other enteric parasites [1]. As shown in Table 1, more than 40% of both diarrheal and normal fecal specimens was found to be positive by the ELISA. On the other hand, *Giardia* parasite was observed in 20.6% of diarrheal feces by microscopic examination after the zinc sulfate centrifugal flotation, indicating higher sensitivity of the ELISA. As one or multiple species of viruses were detected in about half of the diarrheal cases, it was unusual to find the case in which *Giardia* was exclusively present but four cases gave *Giardia* only.

In the present survey two notable epidemiological features was observed; one was a distinct drift of CPV-2 antigenic type after the previous survey in 1997, and the other was high prevalences of CCV and *Giardia* infections.

Three antigenic types 2, 2a, and 2b have been historically recognized by monoclonal antibodies for CPV-2 which emerged since 1978 [20], and recently new antigenic type 2c strains have been discovered from wild cats in South-east Asia [9]. The present survey showed that type 2b has become the predominant antigenic type of CPV-2 in Japan, and this was different from our previous result; of 20 CPV-2 isolates obtained in 1997, a half were 2a and the remaining half were 2b (unpublished data). It has been believed that the original antigenic type 2 disappeared in early 1980's from the field [20]. Thus, the original antigenic type 2 isolate detected in this study probably have originated from canine vaccine products currently used in this country, which universally employ the original type 2 viruses.

Although it has been generally thought that infections of CCV and *Giardia* may cause either mild to moderate clinical disease or asymptomatic infections in dogs, more severe disease may occur in younger animals [2, 11]. The present

observation that they were frequently detected even in normal feces also suggests feeble pathogenic potency of those agents, especially when compared with highly pathogenic agents such as CPV-2 exclusively detected in the diarrheal feces. The present detection rate of CCV in the diarrheal feces was obviously higher than the latest prevalence rate (16%) obtained in 1997 [5], indicating more extensive dissemination of CCV among dogs in Japan. The present data again showed a high prevalence of CCV among dogs in Japan as previously described [5, 22].

It has been recognized epidemiologically as well as clinically that *Giardia* is sometimes observed in stool specimens from dogs with or without diarrhea. In some occasion it causes a serious clinical problem in dogs, especially puppies [21]. *Giardia* species can be referred to as a single species, *G. lamblia*, also known as *G. intestinalis*. It has been well known that *Giardia* is the most common human intestinal parasite, and domestic as well as wild animals may act as reservoirs of human infection, indicating zoonotic nature of giardiasis [11]. In dogs, the prevalence of *Giardia* in the United States, Australia, the United Kingdom and Japan has been reported to be between 1% and 39% [3, 4, 6, 11, 23]. In the present survey, microscopically 23.4% and serologically 48.2% of the stools were found to contain *G. lamblia* and/or the specific antigen associated with *Giardia* infections. The prevalence rate obtained here was slightly higher than the previous reports in Japan [3, 4] and the positive results were almost equally obtained from both diarrheal and normal fecal samples. We could not determine the pathogenic potential of *Giardia* based on only the present epidemiological result, and it may not be enough sample numbers to get a conclusion. However, the data clearly indicated that *G. lamblia* is a common enteric parasite of dogs even at present. Further studies are required to elucidate the role of this ubiquitous protozoan parasite from the viewpoint of canine epidemiology as well as zoonosis.

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