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NOTE

A serological survey of minute virus of canines (MVC ; Canine parvovirus type-1) in dogs in the Tokai area of Japan

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Abstract

A serological survey for antibodies to minute virus of canines (MVC) by use of a hemagglutination-inhibition (HI) test was performed on sera collected from dogs in the Tokai area of Japan. Forty-one of 266 (15.4%) sera had positive titers of 1 : 40 or higher against the MVC. Results suggest that MVC may have been present in dogs in Japan since, at least, 1990. From this serosurvey, MVC appears to be established in the dog population in Japan. MVC may have a role as a newly recognized viral pathogen of dogs in Japan.

Key words : dog, minute virus of canines, serological survey

Canine parvovirus type 1(CPV-1), named the minute virus of canines(MVC), was originally isolated in 1967 from the feces of normal dogs[3]. Physical and chemical properties of MVC are typical of parvoviruses[5]. However, MVC is antigenically distinct from other parvoviruses, including canine parvovirus type 2 (CPV-2) which is well known as a causative agent of world wide pandemic of severe hemorrhagic enteritis and myocarditis in dogs since 1978[1,2,5,9].

MVC is capable of producing subclinical to fatal enteritis and lymphadenitis in neonatal pups, and experimental studies have shown that MVC may cause mild to severe pneumonitis and enteritis in neonatal pups as well as embryo resorptions or fetal death in pregnant bitches[5,14].

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Principal histopathological lesions in infected pups and fetuses are observed in the lung, small intestine, lymph nodes and, in some cases, the heart. Characteristic lesions include interstitial pneumonia with basophilic intranuclear inclusion bodies in the bronchial and alveolar epithelial cells, as well as in epithelial cells at the tips of intestinal villi in the duodenum and jejunum[5,6].

Studies on the seroprevalence of MVC suggest that this virus is widespread in the dog population in the United States[5,7]. Recently, natural cases of MVC infection in pups and fetuses were reported in Sweden[13], Germany[17]and Italy[16]. MVC infections in Japan have not been recognized until recently. In this paper, we report the seroprevalence of MVC in the dog in Tokai area of Japan.

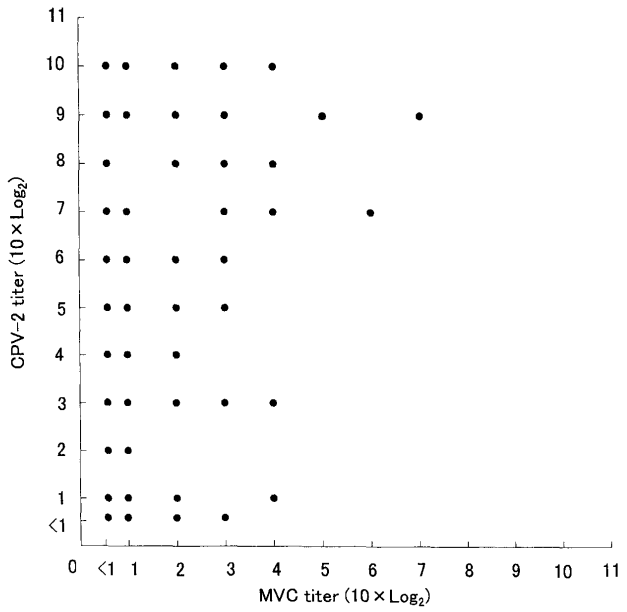
A total of 266 serum samples were collected from dogs in several areas in Aichi and Gifu prefectures during a period from January to June of 1990. Ages of dogs were between 2 months and 8 years, and the average age was 2.2 years. There were 152 male dogs, 111 females and 3 were of unknown sex. Serum samples were stored at −80℃ until tested.

All serum samples were tested for both anti-MVC and -CPV-2 antibodies by hemagglutination-inhibition (HI) tests. Serum samples for HI tests were heated at 56℃ for 30 min. A 1 : 10 dilution of each serum sample was made in phosphate-buffered saline solution. The diluted serum then was absorbed with a 50% (v/v) suspension of *rhesus macaque* erythrocytes, mixed well and incubated for at least 2 hr at room temperature, or overnight at 4℃. Erythrocytes were then removed by low speed centrifugation. Two-fold serial dilutions were made in V-bottom plastic plates, using 0.025 ml droppers and diluters. To each serum dilution was added 0.025 ml of hemaggutination (HA) antigen that contained 4 to 8 HA units of either MVC or CPV-2. After standing for 1 hr at room temperature, 0.05 ml of the ice-chilled erythrocyte suspension was added. HI titers were recorded as the reciprocal of the highest dilution with >75% HA -inhibition. HI test for CPV-2 were performed according to the procedure described previously[4].

Table 1. Results of tests for HI antibodies to MVC and CPV-2 of dogs

Titer	MVC No. of dogs	CPV-2 No. of dogs	Classification
<10	101	52	Negative
10	94	4	〃
20	30	12	〃
40	18	11	Positive
80	13	23	〃
160	6	25	〃
320	1	10	〃
640	2	24	〃
1,280	1	46	〃
2,560	0	29	〃
5,120	0	15	〃
≥10,240	0	15	〃

Total sera : n=266



Titer ≥ 1:40 is regarded as seropositive.

Fig. 1. HI titers of serum samples of dogs with MVC and CPV-2

Results of HI tests on 266 sera are given in Table 1 and Figure 1. Determination of "positive" sera is based on the results described previously[7]. Briefly, tests of 88 control (negative) sera from SPF Beagles from Baker Institute's collony revealed that 88% had titers<1 : 10 ; 10% had titers of 1 : 10, and 2% titers of 1 : 20. Because the dogs had not been exposed to MVC and CPV-2, those titers were considered nonspecific. Only titers of ≥1 : 40 were regarded as seropositive. Based on this criterion, 41 of 266 sera (15.4%) had positive titers for MVC. On the other hand, 198 of 266 sera (74.4%) had positive titers for CPV-2, indicating highly prevalence of CPV-2 antibody. There have not been any cross-reaction in the HI test with different parvoviruses and it has been shown that MVC does not cross-react with CPV-2s [5,14]

Results of this survey for anti-MVC antibodies suggest that the virus may have been present in the Japan dog population since at least 1990, although tests were done in limited areas. Seroprevalence rates of MVC, based on HI tests in the United States, have been reported to be between 30% and 70% [5,7]. The positive rate obtained from the present survey was relatively low compared with that of the United States. Results reported here appear to be significant ; however, sample numbers were insufficient to allow a explicit conclusion. The present serological tests of dogs in the Tokai area also revealed a high prevalence of CPV-2 antibodies. Thirty dog had titers of 5, 120 to>10, 240, which suggests prior infections with CPV-2, although clinical data for those dogs were lacking. There is no evidence whether dual infections with CPV-2 and MVC had occurred. Antigenic and genomic properties and also pathogenicity of CPV-2 are distinct from MVC[5,14]. CPV-2 infection emerged in 1979 in Japan[2] and it seemed to be widespread in dog population in Tokai area at the time of serological survey of MVC performed[11]. On the other hand, MVC was first isolated in the United States from the feces of normal dogs in 1967 and it seems reasonable to assume that the virus spreaded similaly to CPV-2 [3,12]. Thus, the presence of anti-MVC HI antibodies appears to be unrelated to CPV-2 infection.

Report on the isolation of MVC is lacking in Japan. The availability of virus isolation methods is limited since *in vitro* cultivation is restricted to the Walter Reed canine cell (WRCC) line, although MVC also may replicate in primary lung cells from dog embryos[6]. Harrison et al.[10] attempted virus isolation using Maden Darby canine kidney (MDCK) cultures, the Crandell feline kidney cell line, the A-72 cell line and WRCC cultures. Cytopathogenic changes were observed only in the WRCC cultures and the presence of MVC in WRCC cultures was confirmed.

Natural infection case of MVC has not yet been reported in Japan ; however, fatal cases

of fetal and pup infections with MVC were reported in several countries[13,16,17]. Recently, Mochizuki et al. were successful for virus isolation and in detecting MVC viral DNA from rectal swab samples from the pups with diarrhea (The 131st Meeting of Japanese Society of Veterinary Medicine, April, 2001. Tokyo). On the other hand, outbreaks of CPV-2 infection have occurred since March 1979, and the first HI positive case was detected in serum samples collected in October 1978 in Japan[15]. This suggests that the natural occurrence of viral infection is closely correlated with results of serological tests. Thus, results of this serological survey, together with the difficulty of MVC isolation, may indicate that MVC infection of neonatal pups may have been overlooked in Japan.

MVC now appears to be established as a cause of non-fatal to fatal illness in young pups and transplacental infections with fetal death, including mummification and embryo resorption, in pregnant dams[6,7]. More than 75% of pup deaths occur prior to the 3rd weeks of life, the vast majority occurring during the first week due to variety of causes, including viral infection[8]. It is also recognized that there is a lack of knowledge of the true cause of most neonatal illness or death. We could not determine the pathogenic potential of MVC in Japan based only on the present seroepidemiological results. However, the present data suggest that MVC infection may have a role in cases of unexpected deaths of fetal and neonatal pups and enteritis and pneumonitis in young pups, or reproductive disease of pregnant dams in Japan.

In conclusion, we demonstrated for the first time that specific HI antibodies to MVC have been found in dogs in Japan. Further studies are required to elucidate the pathogenic role of MVC in cases of neonatal pup mortality and peri-natal diseases of pregnant dogs.

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