Seroepizootiologic Studies on Rotavirus Infections of Dogs and Cats

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ABSTRACT. Seroepizootiologic survey among dogs and cats in the South-Kyushu area and serological tests for diagnosis of rotavirus infections were investigated by using canine RS 15 and porcine S80 strains. Although no clinical sign was observed in the puppies orally inoculated with RS 15 strain, all of them mounted an immune response and seroconversion within a week or so was detected by plaque reduction neutralization test (PRNT), hemagglutination-inhibition (HI) and reverse passive HI (RPHI) tests. It took more than 2 weeks to detect complement-fixation (CF) antibody in their sera. HI test was applied for detection of type-specific antibody instead of PRNT because antibody titers obtainable by both tests were generally correlative. RPHI test was considered to be applied routinely for serological diagnosis of rotavirus infections of dogs and cats. CF, RPHI and HI antibodies against RS 15 strain were detected in 25.0%, 42.1% and 41.9% of dog serum samples, respectively. About 60% of antibody-positive sera detected in the dogs were considered to be derived from infections with the same serotype as RS 15 strain. Although rotavirus antibodies were detected in the cats as well, it was supposed that different serotype(s) might have been epizootic in the population. HI antibody against RS 15 strain was detected in 13.8% of human sera.—KEY WORDS: cat, dog, reverse passive HI, rotavirus infection.


Viral enteritides of dogs and cats as companion animals are of consequence in veterinary clinics as well as in zoonotic implications. With the exception of the viruses which are highly pathogenic to alimentary tract, canine and feline parvoviruses for instance, the pathogenic significance of other enteric viruses and virus-like particles detected in the feces of these animals with and without diarrhea has not been well understood [2, 6, 10, 18]. In man and domestic animals, rotavirus is a major enteric pathogen which has been intensively investigated, but many aspects concerning rotavirus infections of companion animals have been left unascertained. The authors have already reported some characteristics of a canine rotavirus isolate (RS 15 strain) [12–14]. This paper describes serological tests routinely applicable for dogs and cats, and serological evidence of rotavirus infections of the animals in the South-Kyushu area.

MATERIALS AND METHODS

Cell culture and media: MA104 cells were used for propagation of viruses and plaque reduction neutralization test (PRNT). The cells were grown in Eagle’s minimal essential medium (Eagle’s MEM) containing 10% calf serum, 10% tryptose phosphate broth and antibiotics (100 U of penicillin G, 100 μg of streptomycin and 2.5 μg of amphotericin B/ml).

Viruses: Canine rotavirus RS 15 strain and porcine rotavirus S80 strain [14] were employed. The viruses were propagated in MA104 cells maintained with Eagle’s MEM which contained no serum but trypsin (SIGMA Chem. Co., type 1) at a final concentration of 10 μg/ml.
Animal inoculation: Three conventional littermate puppies were inoculated orally with RS 15 strain. They were about 4–5 weeks old, clinically healthy and seronegative as determined by complement-fixation (CF), hemagglutination-inhibition (HI) and reverse passive HI (RPHI) tests before inoculation. Each puppy received $6.8 \times 10^7$ PFU of the virus and 4 weeks later they were reinoculated with $1.7 \times 10^7$ PFU. While they were observed for clinical signs feces were collected daily, and sera weekly for 9 weeks. Virus shedding in feces was tested by reverse passive hemagglutination test as reported previously [13].

Field serum samples: One hundred and seven dog serum samples were collected during the time from 1981 to 1985, and 92 cat serum samples during the time from 1982 to 1985. A total of 66 human sera were collected during the time from 1984 to 1985: 26 samples from students and staffs, aged 21–33, in the Department of Veterinary Medicine and 40 samples from the clinically healthy people, aged 19–37, of Kagoshima City. Thirty-seven persons of the latter group had kept no animal in their home.

Serology: The methods of CF, RPHI, HI and PRNT were the same as those reported previously [8, 14, 19, 24]. The supernatant fluid of infected MA104 cell cultures was used as antigen in CF test and PRNT, and the partially purified virus in RPHI and HI tests. The virus was purified by CsCl density gradient centrifugation as described previously [13, 14].

RESULTS

Serological response of puppies inoculated with RS 15 strain: No clinical sign was observed in the puppies and viral shedding in feces was detected only on the next day after the first and second inoculations (Fig. 1). Preinoculation sera of the puppies had some virus-neutralizing activity, yet no antibody was detected by CF, RPHI and HI tests. Rapid rise in antibody titers was detected in all the puppies on the 7th day after inoculation by PRNT and HI test with PRNT titers being 10 times or more higher than HI titers. In spite of the marked PRNT and HI antibody response, CF antibody was first detected in one puppy on the 14th day after inoculation but 1 to 2 weeks later in other animals. RPHI antibody appeared more rapidly and with higher titers than CF antibody. Distinct secondary antibody response to the second inoculation was detected by PRNT and RPHI test.

Rotavirus antibodies in dog, cat and human sera: As 40% of dog and 27% of cat serum samples possessed anticomplementary activity, CF antibody in these sera could not be determined. As shown in Table 1, 25% and 9% of dog and cat serum samples, respectively, had CF antibody against RS 15 strain. CF antibody against porcine rotavirus S80 strain was also detected in the sera and its relation to the antibody against RS 15 strain in each sample is exhibited in Fig. 2. There were some dog serum samples which had CF antibodies reactive to respective strains alone, but no cat serum sample possessing CF antibody to only S80 strain was found. All seropositive cat sera had CF antibody reactive to RS 15 strain and the prevalence rate of CF antibody to S80 strain was very low in the cat.

RPHI antibody against RS 15 strain could not be determined in the sera with nonspecific agglutinability which existed in about 29% of both the dog and cat samples. The antibody was positively detected in 42.1% of dog and 31.8% of cat serum samples as shown in Table 1, and the prevalence rates, especially in cats, were higher than those determined by CF test. The relationship between antibody titers obtained by CF and RPHI tests is shown in Fig. 3. Both antibody titers in dog and human serum samples were generally cor-
relative and the calculated correlation coefficients were found to be $r=0.80$ and $r=0.72$, respectively. However, RPHI antibody was positively detected in many cat sera without CF antibody ($r=0.40$).

HI titers compared with PRNT titers in selected dog sera are shown in Fig 4. Both antibody titers were correlative ($r=0.79$), but HI titers were lower than PRNT titers 10-fold or more and all serum samples possessed virus-neutralizing activity. HI antibody against RS 15 strain was detected in 41.9% of dog serum samples, but it was demonstrated in only 1 of 91 cat serum samples. The ratios of HI antibody-positive to RPHI antibody-positive samples were 59.4% and 5% in the dog and cat, respectively.
Table 1. Prevalence of antibodies against canine rotavirus RS 15 strain in dog, cat and human sera

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of sample tested</th>
<th>CF&lt;sup&gt;a&lt;/sup&gt; test with RS 15 strain</th>
<th>CF&lt;sup&gt;a&lt;/sup&gt; test with S80 strain&lt;sup&gt;d&lt;/sup&gt;</th>
<th>RPHI&lt;sup&gt;b&lt;/sup&gt; test with RS 15 strain</th>
<th>HI&lt;sup&gt;c&lt;/sup&gt; test with RS 15 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>107</td>
<td>16/64 (25.0%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12/59 (20.3%)</td>
<td>32/76 (42.1%)</td>
<td>44/105 (41.9%)</td>
</tr>
<tr>
<td>Cat</td>
<td>92</td>
<td>6/67 (9.0%)</td>
<td>1/64 (1.6%)</td>
<td>21/66 (31.8%)</td>
<td>1/91 (1.1%)</td>
</tr>
<tr>
<td>Human</td>
<td>66</td>
<td>51/66 (77.3%)</td>
<td>NT&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38/65 (58.5%)</td>
<td>9/65 (13.8%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Complement-fixation.<br/>
<sup>b</sup> Reverse passive hemagglutination-inhibition.<br/>
<sup>c</sup> Hemagglutination-inhibition.<br/>
<sup>d</sup> Porcine rotavirus which exhibits different antigenicity from RS 15 strain by CF test.<br/>
<sup>e</sup> Number of positive/number of tested without any non-specific inhibition (incidence rate of positive sample).<br/>
<sup>f</sup> Not tested.

![Graph showing CF titer against RS 15 strain for DOG and CAT](image)

Fig. 2. Complement-fixation (CF) antibody against canine rotavirus RS 15 strain and porcine rotavirus S80 strain in dog and cat sera.

CF, RPHI and HI antibodies to RS 15 strain were detected in 77.3%, 58.5% and 13.8% of human serum samples, respectively, and there was no significant differences in the titer and prevalence of antibodies in each human group as shown in Fig. 3.

DISCUSSION

Rotavirus has been widely recognized as the primary cause of enteritides in the youngs of man and domestic animals, and severity of clinical disease is age-dependent. Although rotaviruses have been certainly isolated from dogs and cats with or without diarrhea [3, 4, 7, 13, 22] and the symptomatic illness has been also recorded in neonatal animals experimentally inoculated with the viruses [9, 22], the studies so far have indicated that mild diarrhea in very young animals and inapparent infections in others are most possible type of the disease, and the rotavirus may not be the principal etiological agent of diarrhea in dogs and cats [1, 3, 7, 11, 15, 21, 26]. The puppies manifested no clinical sign and the duration...
Fig. 3. Complement-fixation (CF) and reverse passive hemagglutination-inhibition (RPHI) antibodies against canine rotavirus RS 15 strain in dog, cat and human sera. In the human samples: ○ Students and staffs of the Department of Veterinary Medicine; ● Clinically healthy people of Kagoshima City.

Fig. 4. Plaque reduction neutralization test (PRNT) and hemagglutination-inhibition (HI) antibodies against canine rotavirus RS 15 strain in dog sera.

of fecal virus shedding was very short compared with that reported by other researchers [1, 9, 21, 26], while all puppies mounted an immune response to the virus and serological tests detected their seroconversion within a week or so, showing that they were definitely infected with RS 15 strain by oral route. The immune response of the puppies was so rapid that it might be anamnestic as observed by Tzipori and Makin [26].

PRNT was considered to be very sensitive, but all sera examined had PRNT antibody titer of more than 1:4. These low neutralizing activities may be non-specific because no activity was found in IgG fraction, obtained by salting out at 33% with neutral saturated ammonium sulfate solution, of some selected sera possessing low neutralizing and no HI activities (data not shown). Most possible explanation is that anti-protease activity of the dog serum may inhibit rotavirus replication in the test [5]. Another interpretation is that even low PRNT antibody titers in dog sera may be
specific because of high sensitivity of the test. In the present study HI test is considered to be of use for detection of type-specific antibody in place of PRNT as suggested previously [12].

RPHI test has been used as a serologic tool which mainly detects subgroup-specific antibody as CF test [19]. However, both subgroup- and type-specific antibodies can be detected by the test, which depends on the nature of antibody and antigen applied. The test is supposed to be available for the wide application. It is considered that RPHI test in the present study detects both antibodies since the antiserum against whole virus [14] and a mixture of single- and double-shelled virus particles as antigen have been used. RPHI antibody was generally correlative with CF antibody in dog and human sera, though the difference of titers was notable; which was in accord with the report by others [19]. However, there was a great discrepancy between RPHI and CF titers of the cat serum samples. A possible explanation is that non-complement-fixing antibody may be produced in cats infected with rotaviruses as pointed out in the cats infected with caliciviruses [16] and the cats immunized with various non-infectious antigens [17]. CF antibody was produced at low titer and it took a long time after infection to be detected in the present study. Therefore, CF test is not considered to be a good diagnostic test, but RPHI test with the paired sera taken 2 weeks or more apart is routinely applicable to the serological diagnosis in dogs and cats.

Since the antigenic classification of rotaviruses epizootic among dogs and cats is unknown at the present, it may be nearly impossible to know entire prevalence rate of rotavirus infection of these animals by serological evidence. However, results by using canine isolate in the present study indicate that approximately 40% and 30% of dogs and cats of the South-Kyushu area, respectively, have been infected with rotaviruses, which implies that rotavirus infections occur commonly in dogs of the area as reported in other parts of Japan previously [20, 23, 25]. The detection of CF antibody against porcine rotavirus S80 strain, which may have another antigenic determinant in addition to determinant(s) of RS 15 strain [14], signifies a possibility of infections with different rotavirus subgroup in the dog population.

The present study may be the first demonstration of rotavirus antibodies in cats of Japan. The seroprevalence in the cats of the area is similar to that examined in Scotland and the United States [7, 22], and the infection appears to be widespread in the cat population as in the dogs. The principal subgroup of rotaviruses epizootic in the cats may be the same as RS 15 strain, but not the same serotype.

HI antibody against RS 15 strain was detected in 13.8% of human serum samples, which might reflect possible antigenic similarity of RS 15 strain to rotavirus strains epidemic among humans of the area; we previously demonstrated one-way antigenic relationship between RS 15 and human Wa strains [12, 14].

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REFERENCES


要約

イヌとネコのロタウイルス感染症の疫学的研究：望月雅美・南京子・坂本経1）（鹿児島大学農学部家畜微生物学講座、1）家畜外科学講座）——イヌロタウイルス RS15株感染仔イヌを用いてロタウイルス感染症血清診断試験法について検討し、南九州地域のイヌとネコの血清疫学調査に応用した。ウイルス經口投与後、仔イヌは臨床症状を発現しなかったが、ブラーク減数中和試験（PRNT）、血球凝集抑制（HI）試験および逆受性血球凝集抑制試験により、约1週間で抗体が陽転したが、補体結合試験では2週間以後に陽転した。仔イヌ感染実験と野外イヌ血清を用いて検討した結果、HI試験はPRNTに代替可能な稀有抗体検出法であることが確認された。これらの方法を応用した疫学調査では、（1）本地域のイヌの約40％はすでにロタウイルスに暴露しており、RS15株血清型ウイルスの感染が主であること、さらに複数サブグループウイルスの感染の可能性があること、（2）ネコでは約30％がすでに抗体を有しており、RS15株と同一サブグループながら異型血清型ウイルスの感染・伝播が開うこと、が示唆された。ヒト血清中にもRS15株に対するHI抗体活性が検出された。