イヌコロナウイルスを用いた微量中和試験によるイヌ・ネコのコロナウイルス抗体検出

誌名	Japanese journal of veterinary science
ISSN	00215295
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巻/号	49巻3号
掲載ページ	p. 563-565
発行年月	1987年6月

Micro-neutralization Test with Canine Coronavirus for Detection of Coronavirus Antibodies in Dogs and Cats

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(Received 1 October 1986/Accepted 20 March 1987)

Jpn. J. Vet. Sci. 49(3): 563–565, 1987

KEY WORDS: coronavirus, micro-neutralization.

The coronaviruses are responsible for wide variety of diseases, in particular respiratory and enteric disorders in mammalian and avian species [7]. During our studies on enteric viruses of dogs and cats, a micro-neutralization test (MNT) with a canine coronavirus (CCV) strain has been developed for detection of coronavirus-neutralizing activity. The purpose of this report is to assess the utility of this MNT for detection of coronavirus antibodies in these animals.

A canine coronavirus, designated 5821 strain, was obtained from Dr. Masayuki Ajiki, Kyoto-Biken Lab., Kyoto. This strain was originally isolated from feces of a puppy died of enteritides manifesting diarrhea and vomiting in 1984, and had been previously passaged 8 times in primary dog kidney (DK/P) cells. The virus was further cultured twice in DK/P cells and once in Crandell feline kidney (CRFK) cells in this laboratory, and stored at -80°C as a stock virus. CRFK cells were used for MNT and plaque reduction neutralization test (PRNT), and were cultured in Eagle's minimal essential medium (Eagle's MEM) containing 10% fetal calf serum (FCS), 10% tryptose phosphate broth (TPB) and antibiotics (100 U of penicillin G, 100 μg of streptomycin and 5 μg of amphotericin B/ml) (Eagle's MEM/10FCS).

Two hundred and ninety-two sera were collected from dogs of the local pounds in Kagoshima Prefecture during September, 1984 to October, 1985. One hundred and forty-six sera, 5 ascites and 1 thoracic fluid were collected from cats during 1982 to 1986: 90 sera from clinically normal cats (group A in Fig. 2), 40 sera from cats with illness other than feline infectious peritonitis (FIP) (depression, anorexia and pyrexia, halitosis and oral ulcerations, anemia, renal disorders, gastrointestinal signs, and respiratory tract signs) (group B), and 22 sera and body fluids from cats clinically suspected, not histopathologically confirmed, as FIP (group C). Most of them were submitted by veterinary practitioners in Kagoshima, Miyazaki, Tokyo, Saitama and Kanagawa area for the examination of feline leukemia virus. Hyperimmune sera to CCV 5821 strain prepared in guinea-pigs and to CCV 1–71 strain prepared in rabbits were obtained from Kyoto-Biken Lab., Kyoto and Department of Veterinary Microbiology, University of Tokyo, Tokyo, respectively.

Micro-virus titration and MNT were performed in 96-wells, flat-bottomed, polystyrene plates (Corning Glass Works, NY). After serial tenfold dilutions were prepared from the stock virus fluid with the Eagle's MEM/10 FCS, 50 μl of aliquot was transferred to four wells per dilution. Then 50 μl of CRFK cell suspension at 8×10^5/ml of cell density in the Eagle's MEM/10 FCS was added per well. The plate was gently tapped for mixing and incubated at 37°C for 5 days in a humidified chamber containing 5% CO_2. The end point was determined by the appearance of cytopathic effect (CPE) under the microscope and the titer was expressed as median tissue culture infective dose per 50 μl ($TCID_{50}$/50 μl). For MNT, 25 μl of serum or body fluid was serially diluted twofold with the Eagle's MEM/10 FCS in wells 1 through 11 of a row in the plate. Well 12 was the serum control which consisted of 25 μl each of the Eagle's MEM/10 FCS and the serum at a 1:4 starting dilution. To each of the 11 wells in the serum titration row was added 25 μl of a virus suspension containing 200 $TCID_{50}$/ 50 μl. The plate was gently agitated for mixing and was incubated at 37°C for 1 hr in the humidified chamber. The second virus titration was carried out at the same time to determine the actual dose of virus present in the test by re-titration of the virus suspension. Then 50 μl of the CRFK cell suspension was added to each well, the plate was mixed and incubated for 5 days. The MNT titer was defined as the reciprocal of the highest serum dilution at which CPE had been suppressed completely. The titer less than 1:4 was regarded as antibody-negative in the study.

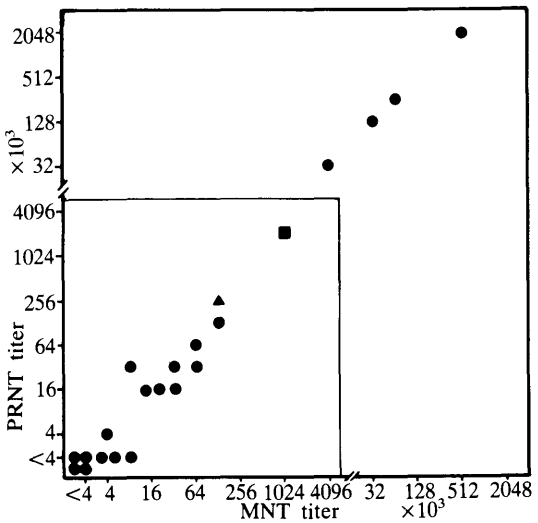

Fig. 1. Comparison between micro-neutralization test (MNT) and plaque reduction neutralization test (PRNT) titers of cat sera and anti-canine coronavirus (CCV) immune sera. ●, Cat serum; ▲, Anti-CCV 5821 strain immune serum; and ■, Anti-CCV 1–71 strain immune serum.

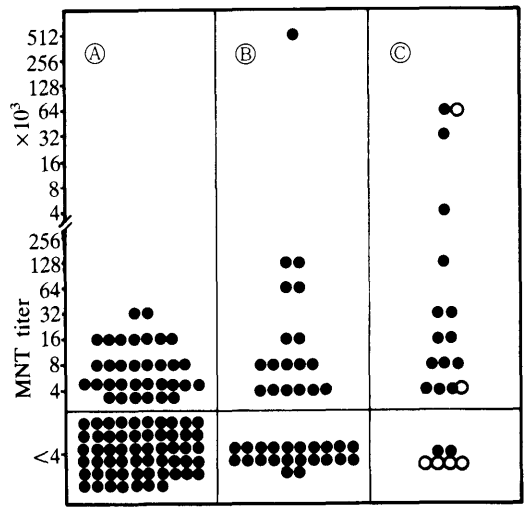

Fig. 2. Micro-neutralization test (MNT) antibody titers against canine coronavirus 5821 strain in serum (●) and body fluid (○) from cats with diverse clinical histories. A=Clinically normal; B=Illness other than feline infectious peritonitis (FIP); and C=Clinically suspected, not histopathologically confirmed, as FIP.

PRNT was performed by mixing equal volumes of twofold serum dilutions and the virus suspension containing 100 plaque forming unit (PFU). After an incubation at 37°C for 1 hr, 0.2 ml each of the mixture was inoculated onto the CRFK cell monolayers in 60-mm Petri-dishes. After 1 hr adsorption at 37°C, excess inoculum was removed, and 4 ml of an overlay medium was added. The overlay medium consisted of the Eagle's MEM containing 2% FCS, 10% TPB, the antibiotics and 0.8% agar noble. The plates were incubated at 37°C for 5 days in the humidified chamber and then stained with the overlay medium containing 0.01% neutral red. The PRNT titer was expressed as the reciprocal of the highest serum dilution which showed median plaque count reduction or more.

Twenty cat sera and the anti-CCV immune sera possessing varied MNT titers were titrated by PRNT. The antibody titers obtained by both methods were generally correlative and the calculated correlation coefficient was found to be r=0.985 as shown in Fig. 1. However, MNT titers were 4–8 times lower than PRNT titers at 1:1,000 or more dilution of serum.

Sixty-seven dog sera (22.9%) possessed anti-CCV MNT antibody titers of 1:4 to 1:256 with the geometric mean (g) antibody titer of 1:16. As shown in Fig. 2, 36.7% of sera from clinically normal cats (group A) were anti-CCV neutralizing antibody-positive and the g antibody titer of the positive sera was 1:7. The antibody positive rate was 45% in the sera from cats with illness other than FIP (group B). The g antibody titer was 1:22 and a serum possessing extremely high antibody titer (1:512,000) was derived from the cat clinically diagnosed as renal disorders. Five serum samples possessing the titer of more than 1:64 in the group B were derived from 2 cats suffering from renal disorders and 3 cats manifesting depression, anorexia, pyrexia and anemia. In the sera and body fluids from cats in the group C, 72.7% of them were positive with the g titer of 1:78. The majority of antibodies detected in the cats by the MNT with CCV 5821 strain may account to infections with either CCV-like strains of FIPV or non-FIP inducing type II feline enteric coronaviruses [5]. Another possibility is the infection with other coronavirus species resulting in antibody production [2, 6].

In conclusion the MNT in the present study is considered to be widely available for serological studies of coronaviruses. It bears comparison with PRNT as regards the sensitivity and reliabil-

ity, and is obviously superior to PRNT in respect of expense and simplicity. It may be opportune to keep a continuous cell line, such as CRFK cells [3, 4], A-72 cells [1] or FC cells [8], adequate to the indicator cells for coronaviruses.

ACKNOWLEDGEMENTS. The authors wish to express their gratitude to Dr. Masayuki Ajiki, Kyoto-Biken Lab., Kyoto, and Prof. Shin-ichiro Konishi, Department of Veterinary Microbiology, University of Tokyo, Tokyo for their gifts of canine coronavirus 5821 strain and its immune serum, and anti-CCV 1–71 immune serum, respectively. This study was supported by a Grant-in-Aid for Scientific Research (No. 61760262) from the Ministry of Education, Science and Culture of Japan.

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要　　約

イヌコロナウイルスを用いた微量中和試験によるイヌ・ネコのコロナウイルス抗体検出（短報）：望月雅美・杉浦里津子・阿久沢正夫[1]（鹿児島大学農学部家畜微生物学講座，[1]家畜内科学講座）――イヌコロナウイルス5821株を用いた微量中和試験（MNT）により，イヌ・ネコのコロナウイルス抗体検出を試みたところ，信頼性と鋭敏度について，プラック減数中和試験に比較して遜色なく，広く応用できることがわかった。