Evaluation of Micro-neutralization Test for Diagnosis of Feline Calicivirus and Herpesvirus Infections

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(Received 6 April 1987/Accepted 1 July 1987)

KEY WORDS: feline calicivirus, feline herpesvirus, micro-neutralization

Feline calicivirus (FCV) infection (FCI) and feline viral rhinotracheitis (FVR) caused by a feline herpesvirus 1 (FHV) are very common respiratory diseases of cats [7, 8]. Clinical signs caused by FCV, FHV, reovirus, mycoplasmas and Chlamydia can be similar to one another, thus the diagnosis of FCI and FVR on clinical grounds alone is uncertain [1]. Serologic diagnosis with paired sera by plaque reduction neutralization test (PRNT), micro-neutralization test (MNT) and hemagglutination-inhibition test are of retrospective nature [2, 4, 6], and a definitive diagnosis of choice is achieved by virus isolation from an oropharyngeal swab in feline cell cultures. Since a combined vaccine product composed of modified live FCV and FHV, and killed feline panleukopenia virus has been recently introduced in Japan, there are increasing needs for testing serum antibody titers against FCV and FHV in our laboratory. The PRNT has been adopted for this purpose, but many serum samples can not be examined at the same time because of its laboriousness and costliness. Herein we present an evidence which shows the MNT for determination of coronavirus antibody of dogs and cats reported previously [5] can be applied as a serologic test of FCI and FVR as well.

Twenty sera randomly selected from cats immunized with the combined vaccine (FVR C-P; Pitman-Moore, Inc., NJ, U.S.A.) were titrated by PRNT and MNT with FCV F9 strain and FHV C7301 strain [3]. The materials and methods of both tests were the same as those reported previously [5]. Briefly, PRNT was performed by mixing equal volumes of serum dilutions and the virus suspension containing 100 plaque forming unit. After incubation at 37°C for 1 hr, 0.2 ml of each of the mixture was inoculated onto CRFK cell monolayers in 60-mm Petri dishes. After 1-hr adsorption at 37°C, 4 ml of an overlay was added. The dishes were incubated at 37°C for 72 hrs in a humidified chamber containing 5% CO₂ and then stained with 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. In the MNT, serum was serially diluted with Eagle's minimal essential medium containing 10% fetal calf serum in wells of a 96-well flat-bottomed plate. Virus prediluted to a titer of 10-32 TCID₅₀/25 µl was then added to each well in a 25-µl amount. The plate was gently agitated for mixing and was incubated at 37°C for 1-2 hrs in the chamber. Then 4 x 10⁴ CRFK cells were added to each well and the plate was incubated for 72 hrs. The MNT titer was defined as the reciprocal of the highest serum dilution at which cytopathic effect had been suppressed completely.

The antibody titers obtained by both PRNT and MNT were generally ccorrelative with each other as shown in Fig. 1. The correlation coefficient between the titers in the case of FHV was slightly lower, and as regards sensitivity,
MNT titers were 3–6 times lower than PRNT titers in the case of FCV, and 2–4 times lower in the case of FHV. In conclusion, the MNT is of practical and useful in the conservation of materials and reagents, as well as time and effort. It should be noted, however, PRNT is recommended when extra sensitivity is required.

REFERENCES


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ネコカリキウイルス及びネコヘルペスウイルス感染診断のための微量中和試験の評価（短報）：望月雅美・赤星隆雄・坂本　総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）——ネコのウイルス性呼吸器病の主原因であるネコカリキウイルスとネコヘルペスウイルスに対する中和抗体測定のため、微量中和試験（MNT）とブラック減数中和試験（PRNT）を比較した結果，MNTはPRNTより銳敏ではないが，簡便・経済的で多数検体処理に適すると思われた。