Reverse passive haemagglutination test for the detection of canine coronaviral antigen

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In the present study reverse passive haemagglutination (RPHA) was employed for the detection of canine coronavirus (CCV) antigen from faecal samples collected from dogs with severe gastroenteritis, and compared with agar-gel immunodiffusion (AGID).

Rectal swabs (86 numbers) were collected aseptically from pet dogs brought to Small Animal Clinic, Madras Veterinary College, Madras, with a history of severe gastroenteritis (with or without vomiting) in 1 ml of phosphate buffered saline (PBS) with 200 IU of penicillin and 50 µg of streptomycin/ml. The charged samples were clarified by centrifugation at 2000 rpm for 10 min. The supernatant was aliquoted and stored at -20°C until used.

Canine coronavirus (1-71), supplied by Dr M J G Appel, James A Baker Institute of Animal Health, New York, USA, was used in the present study as reference virus and for the preparation of antiserum.

The reference CCV was propagated in Crandell feline kidney (CrFK) cell-line supplied by the National Facility for Animal Tissue and Cell Culture, Pune, India, to increase antigenic mass for hyperimmunization work. The anti-CCV serum was raised in rabbits (Reynolds \textit{et al.} 1980). The cell-culture fluid harvested was concentrated 50-folds by precipitating with 40% saturated ammonium sulphate. The precipitate was then dissolved in glass-distilled water and pelleted at 100 000 g (Beckman L7-80 Centrifuge, 70Ti Rotor) for 1 hr. The pellets were suspended in 0.15 M NaCl and emulsified with equal volume of Freund's incomplete adjuvant. Antigen was administered in 1 ml quantities intramuscularly thrice at an interval of 14 days. The serum was collected aseptically after 7 days of last injection, tested for the presence of CCV antibodies and stored at -20°C until use.

The immunodiffusion test was performed on glass-slides with 0.8% noble agar (Thanappa Pillai \textit{et al.} 1990). The reference CCV antigen was used as positive control along with a known negative control.

Reverse passive haemagglutination test was essentially the same as described for rinderpest (Scott 1991). Sheep blood was collected in Alsever's solution and washed with PBS (pH 7.2) thrice and finally 2.5% of red blood cells (RBC) was prepared in PBS. The RBCs were fixed with glutaraldehyde by mixing 25 ml of 2.5% of RBC suspension with 3 ml of 2.5% glutaraldehyde in PBS and stirring gently at 37°C for 1 hr, till the RBCs changed from red to brown colour. The fixed RBCs were triple washed at 500 g for 15 min in PBS and a stock of 2.5% concentration of packed fixed RBCs was made. Sodium azide (0.02%) was added as a preservative and stored at +4°C. Antibody labelling was performed by adding 1.0 ml of 1 in 10 dilution of the known antiserum to 3.0 ml of PBS to which 1 ml of 2.5% fixed RBCs was added.
They were mixed thoroughly at 37°C for 30 min. The antibody labelled fixed RBCs were washed thrice in PBS and resuspended in 5 ml PBS.

The RPHA was carried out in V-bottom plates using PBS as diluent. Two-fold serial dilution of the test antigen (50 µl) was made. This was followed by the addition of 50 µl of antibody labelled, fixed RBCs to all the wells, mixed and incubated at 37°C. The plates were examined after 60 min for haemagglutination. The RPHA titre was expressed as the reciprocal of the highest dilution giving haemagglutination. A RPHA titre of 1:8 was taken as positive, as non-specific agglutination was produced by some of the negative samples wherein the titres never exceeded 1:4.

The significance of RPHA and AGID was worked out using Student’s ‘t’ test (Snedecor and Cochran 1967).

Out of the 86 samples screened 34 samples (39.5%) were positive by RPHA while 14 samples (16.2%) were positive by AGID. Student’s ‘t’ test revealed that RPHA was highly significant when compared to AGID ($t = 3.3039^{**}; P<0.01$).

The results obtained by AGID are corroborative to the previous study (Thanappa Pillai et al. 1990) where 17.1% of samples were positive. The RPHA test was, however, performed for the first time for the detection of CCV antigen from the faecal swabs.

It is suggested that RPHA is a simple and sensitive technique which can be used as a spot diagnosis for coronavirus infection in dogs. It is also helpful in quantification of the antigen.

REFERENCES


