DIFFERENTIATION OF CANINE CORONAVIRUS AND PORCINE TRANSMISSIBLE GASTROENTERITIS VIRUS BY NEUTRALIZATION WITH CANINE, PORCINE AND FELINE SERA

D.J. REYNOLDS, D.J. GARWES and S. LUCEY

A.R.C. Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks RG16 0NN (Great Britain)

(Accepted 14 July 1980)

ABSTRACT

Reynolds, D.J., Garwes, D.J. and Lucey, S., 1980. Differentiation of canine coronavirus and porcine transmissible gastroenteritis virus by neutralization with canine, porcine and feline sera. *Vet. Microbiol.*, 5: 283—290.

Monospecific antisera were prepared in rabbits against canine coronavirus (CCV) and transmissible gastroenteritis virus of pigs (TGEV), and in 24 pigs and 3 cats against TGEV alone. Neutralizing antibody titres were higher for the immunizing than the heterologous virus, although cross-neutralization usually was detected. This confirmed that CCV and TGEV are distinct, but antigenically related coronaviruses. In sera from 41 dogs, CCV-neutralizing titres were on average 2.7 fold higher than TGEV-neutralizing titres, suggesting that CCV was the causal agent. Sera from 29 cats in colonies with feline infectious peritonitis (FIP) and known to contain TGEV-neutralizing antibody, were found to have titres 12.3 fold higher against CCV. The FIP virus (FIPV) is probably more closely related to CCV than TGEV as judged by antigens involved in virus neutralization.
   Antisera to two isolates of bovine coronavirus, three isolates of haemagglutinating encephalomyelitis virus, seven strains of avian infectious bronchitis virus and the 229E strain of human coronavirus all failed to neutralize CCV and TGEV. Thus CCV, TGEV and probably FIPV fall into a group of antigenically related agents, separable from other members of the family Coronaviridae, by both virus neutralization and immunofluorescence tests.

INTRODUCTION

   A close relationship between canine coronavirus (CCV) and the coronaviruses of porcine transmissible gastroenteritis (TGEV) and feline infectious peritonitis (FIPV) has been demonstrated by immunofluorescence tests (Pedersen et al., 1978). Virus neutralization (VN) tests with FIPV itself have not yet been possible owing to the lack of a simple, readily available test. However, some cross neutralization between CCV and TGEV has been demonstrated using convalescent sera from CCV-infected puppies (Binn et al., 1975). Sera from dogs and cats in Britain have VN antibodies for TGEV

(Cartwright, 1973; Reynolds et al., 1977), yet, although TGEV can infect both species experimentally (Larson and Morehouse, 1976; Reynolds and Garwes, 1979), the frequency with which this occurs is probably very low as TGEV is uncommon in pigs in the UK (Wood, 1979).

In this paper the ability of VN tests to differentiate TGEV and CCV has been investigated by production of specific antisera in different hosts. Homologous and heterologous VN titres in antisera to known viruses were then compared with titres in sera from populations of dogs and cats, in which the agent responsible for antibody development was unknown. The objective of this work was to determine the feasibility of serological distinction between possible TGEV infection and CCV or FIPV infection of dogs or cats respectively. This distinction would be important in any epidemiological investigation of the role of dogs and cats in TGEV outbreaks, using serology alone, since both species would be expected to possess antibody to their own respective viruses.

The relationship of CCV and TGEV to other representative coronaviruses was examined by VN tests. Studies have been published (Pedersen et al., 1978) which indicated that by immunofluorescence tests at least two unrelated groups exist. Antisera were obtained from within each group for this study. Antisera to avian infectious bronchitis virus (IBV), bovine coronavirus (BCV) and haemagglutinating encephalomyelitis virus (HEV), which did not react in fluorescence tests with CCV or TGEV antigens, were tested for neutralization. Antiserum to a human coronavirus (229E), previously reported by Pedersen et al. (1978) to react with TGEV and FIPV, was also tested.

MATERIALS AND METHODS

Viruses

Canine coronavirus strain 1-71 (Binn et al., 1975) was obtained from the American Type Culture Collection, Maryland, USA. The virus was passaged twelve times in secondary dog kidney (DK/2) cells. Miss S. Cartwright supplied the FS772/70 isolate of TGEV, which was grown in secondary adult pig thyroid (APT/2) cells. British and American isolates of BCV were obtained from Dr. J. Bridger and Dr. N. Zygraich respectively and were grown in primary cultures of calf kidney cells. HEV strains FS, VW and 67N were obtained from Miss S. Cartwright, Professor M. Pensaert and Dr. W. Mengeling, respectively.

Antisera

Rabbits
Cell culture fluids infected with CCV or TGEV were concentrated 50-fold by precipitation with 40% saturated ammonium sulphate (Garwes and

Pocock, 1975). The precipitates were dissolved in distilled water and pelleted at 100,000 × g for 1 h. Pellets were suspended in 0.15 M NaCl and emulsified with an equal volume of Freund's incomplete adjuvant. Uninfected DK/2 and APT/2 cell control antigens were also prepared. This material was used to inject rabbits which were free of CCV and TGEV antibodies. Antigen was administered intramuscularly on three occasions at intervals of 14 days and VN titres were monitored regularly.

Antisera to BCV and HEV were prepared in rabbits in a similar way. The viruses were first purified by sucrose gradient centrifugation (Pocock and Garwes, 1977).

Components of TGEV were used to immunize rabbits as previously described (Garwes et al., 1978/1979). Antiserum to human coronavirus 229E was supplied by Dr. M. MacNaughton.

Pigs

TGEV was used to immunize 24 gnotobiotic and conventional animals by parenteral injection of adjuvanted virus or by oral infection followed by injection.

Cats

Specific antisera to TGEV were produced in three cats after oral infection. One animal was hyperimmunized intraperitoneally as described previously (Reynolds and Garwes, 1979).

Serum samples were collected from 29 clinical cases of feline infectious peritonitis (FIP) in a cat colony and from cats in contact with the infection (Reynolds et al., 1977).

Dogs

Healthy animals up to 12 weeks old, sacrificed for production of primary cell cultures, were used to collect 52 canine sera. The animals originated from a wide geographical area within the UK.

Chicken

Antisera to seven strains of IBV were supplied by Dr. J.H. Darbyshire. The strains were T, Massachusetts, Connecticut, Holte, Gray, Iowa 97 and Iowa 609.

Virus neutralization (VN) tests

TGEV neutralizing antibodies were assayed against 100 TCD_{50} of virus using APT/2 cells in the microtitre method (Witte, 1971). All sera were heat inactivated at 56°C for 30 min before use and complement was not added. Suspensions of DK/2 cells were used in a similar test, developed to assay CCV antibody. End points were determined by indirect immunofluorescence and titres expressed as the log_{10} of the reciprocal of the end-point dilution, calculated by Kärber's method (Kärber, 1931).

Statistical analysis

Of the 52 canine sera, 11 failed to neutralize either CCV or TGEV. The results for the remaining 41, and for sera from the 24 TGEV-immunized pigs and the 29 cats with FIP, were plotted (Fig. 1) using the $\log_{10}$ titres against TGEV and CCV as the x and y axis respectively. Analysis of variance was used to see if the three populations of sera could be distinguished by separate regression lines. The regression analysis had the following analysis of variance structure:

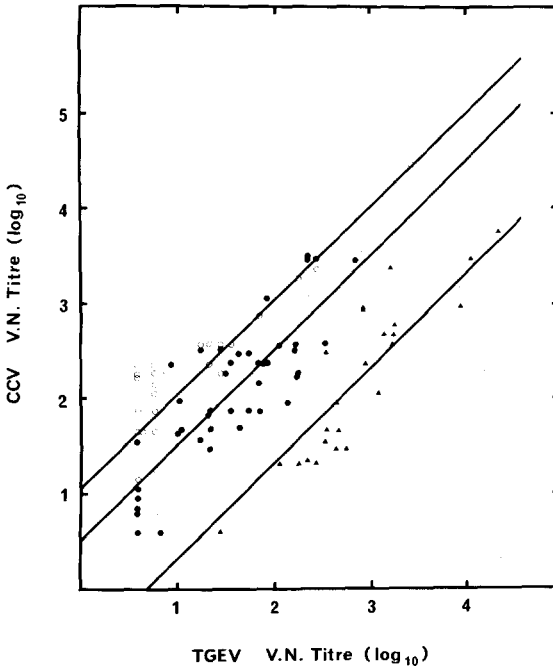| Source of variation	Degrees of freedom	
(1) Common regression line	1	
(2) Deviations from common regression line	4	
a) Displacement		2
b) Lack of parallelism		2
(3) Residual	88	

Fig. 1. CCV and TGEV VN titres, expressed as $\log_{10}$ of reciprocal of endpoint dilution values, for sera from three populations of animals. ▲ — porcine sera from TGEV-vaccinated animals; ● — canine sera from healthy dogs; ○ — feline sera from FIP-infected animals.

RESULTS

Parenteral immunization of two rabbits each with CCV or TGEV resulted in high homologous VN titres as shown in Table I. The rabbits immunized with uninfected DK/2 or APT/2 cells did not develop VN antibody. Neutralization of the heterologous virus occurred with each antiserum but the titres were at least ten-fold higher for the immunizing virus. As previously reported (Garwes et al., 1978/1979) serum from rabbit 5, immunized with TGEV surface projections, resulted in TGEV neutralizing antibody. CCV was also neutralized but with a sixteen-fold lower titre. Neither CCV nor TGEV were neutralized by serum from the rabbit immunized with TGEV subviral particles.

TABLE I

VN titres of sera from rabbits immunized with CCV or TGEV

Immunizing virus	Rabbit number	VN titre[a]	
		CCV	TGEV
CCV	1	2.95	1.95
	2	4.10	2.95
TGEV	3	1.15	2.45
	4	1.65	2.85
TGEV SP[b]	5	1.45	2.65
TGEV SVP[b]	6	<0.6	<0.6

[a]Expressed as $\log_{10}$ of the reciprocal of the end-point dilution.
[b]SP (surface projections); SVP (subviral particles).

The results of infection and hyperimmunization of cats with TGEV are shown in Table II. All three cats were seronegative when first infected. In two cats convalescent sera which had $\log_{10}$ TGEV VN titres of 2.05 and 2.25 did not neutralize CCV. The TGEV VN titre of 2.55 in cat 3, however, did neutralize CCV to a titre of 1.56, approximately 10-fold lower. After hyperimmunization with TGEV, this cat showed a 100-fold increase in titre to both viruses.

Means and ranges of VN, CCV and TGEV titres in serum samples collected from cats, dogs and pigs are shown in Table III. The regression analysis carried out on titres for the canine, porcine and feline sera showed that separate regression lines could be fitted to each of the populations (Fig. 1). These lines were found to be significantly different in their displacement ($P < 0.001$), yet all could be considered parallel. Comparisons between two lines showed that the intercepts for the canine and feline sera were both

TABLE II

Serological responses of cats infected with TGEV

Cat number	Days after infection	VN titre[a]	
		CCV	TGEV
1	0	<0.6	<0.6
	94	<0.6	2.05
2	0	<0.6	<0.6
	94	<0.6	2.25
3	0	<0.6	<0.6
	93	1.56	2.55
	128	3.55	4.55

[a]Expressed as $\log_{10}$ of the reciprocal of the end-point dilution.

TABLE III

Summary of VN titres for canine, feline an'd porcine sera

	No.	$\log_{10}$ VN titre		Correlation coefficient between titres against CCV and TGEV
		CCV mean (range)	TGEV mean (range)	
Cats	29	2.42 (0.78—5.35)	1.33 (0.60—3.65)	0.90
Dogs	41	2.03 (0.60—3.45)	1.59 (0.60—2.85)	0.85
Pigs	24	2.18 (0.60—3.75)	2.88 (1.45—4.35)	0.89

significantly different from the porcine sera ($P < 0.001$), and were also significantly different from each other ($P < 0.001$). The three regression lines had the following equations:

Cats $y = 1.09 \ (\pm \ 0.11) + 1.00 \ (\pm \ 0.06)x$
Dogs $y = 0.44 \ (\pm \ 0.11) + 1.00 \ (\pm \ 0.06)x$
Pigs $y = -0.72 \ (\pm \ 0.18) + 1.00 \ (\pm \ 0.06)x$

These lines had a slope of unity. For a given titre against TGEV, therefore, CCV titres were respectively 12.3 (antilog 1.09) and 2.7 (antilog 0.44) fold higher on average for the feline and canine sera and 5.2 (antilog 0.72) fold lower for the porcine sera. The correlation coefficient for each of the equations is given in Table III.

No neutralizing activity was detected in antisera to BCV, HEV, IBV or 229E against either TGEV or CCV; these were all screened at a dilution of 1 in 2.

DISCUSSION

CCV and TGEV have been shown to be related by VN tests, and hyper-immune sera are capable of two-way cross neutralization. Titres of the sera prepared against each agent were higher in homologous than in heterologous neutralization tests. Similarly antiserum to TGEV surface projections neutralized CCV, but only to low titre. Thus, whilst the antigenic relationship detected by virus neutralization resides within the polypeptide of the surface projection, the difference in titres suggests that VN antibodies in animal sera due to TGEV or CCV could be distinguished. Experimental infection of cats with TGEV resulted in VN antibodies with little or no activity for CCV (Table II) or FIPV (Reynolds and Garwes, 1979). However, coronavirus antibody in FIPV-infected cats neutralized CCV more effectively than TGEV. The origin of TGEV VN antibody in British cats is still unexplained. It may result from infection and antigenic stimulation with CCV or FIPV but the results included in this paper indicate that TGEV infection itself cannot be incriminated. The potential for CCV to infect cats in quite unknown.

The occurrence of VN antibodies for TGEV in sera from British dogs has been described before (Cartwright and Lucas, 1972). The absence of a British isolate of CCV has prevented previous serological studies on the origin of this antibody. As TGEV can infect puppies, the possibility has existed that VN antibodies in canine sera were due to infection of dogs with TGEV (McClurkin et al., 1970). Comparison of CCV and TGEV antibody titres in canine sera and sera from pigs immunized with TGEV, however, revealed a significant difference between the two populations (Fig. 1). Thus the TGEV titres observed in dogs may have been the result of cross-neutralization with CCV, due to common external antigens. This is some evidence, therefore, of a natural CCV infection in British dogs.

CCV and TGEV can be clearly distinguished serologically. Biological differences have been detected in vitro, in particular the complete failure to detect growth of CCV in APT/2 cells, whilst TGEV will grow in DK/2 cells (D.J. Reynolds, unpublished results, 1979). This observation correlates with the in vivo potential for TGEV infection of dogs, whereas CCV would not infect piglets experimentally (Binn et al., 1975). Biochemically, CCV and TGEV showed close similarities but with reproducible differences in the polypeptide patterns (Garwes and Reynolds, 1980).

Failure of BCV and HEV antisera to neutralize CCV or TGEV was expected as these agents were unrelated by previous immunofluorescence tests (Pedersen et al., 1978). In that study, fluorescein-labelled 229E antiserum reacted with TGEV but not with CCV-infected cells, but the 229E antiserum used in this work failed to neutralize either CCV or TGEV. Fluorescein-labelled IBV antisera did not react with cells infected with CCV or TGEV (D.J. Reynolds, unpublished results, 1979) or neutralize these viruses.

Our work confirms immunofluorescence data, suggesting antigenic groups within the family Coronaviridae, by describing cross neutralization between TGEV, CCV and FIPV and a failure to detect neutralization using BCV, HEV, IBV and 229E antisera.

ACKNOWLEDGEMENTS

REFERENCES

Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R.H. and Strano, A.J., 1975. Recovery and characterization of a coronavirus from military dogs with diarrhoea. In: Proceedings 78th Ann. Meeting, U.S. Anim. Health Assoc., Roanoke, VA, Oct. 1974, pp. 359—366.
Cartwright, S.F., 1973. A vomiting and diarrhoea syndrome in dogs. In: C.S.G. Grunsell and F.W.G. Hill (Editors), The Veterinary Annual. John Wright & Sons, Bristol, pp. 194—195.
Cartwright, S.F. and Lucas, M.H., 1972. Vomiting and diarrhoea in dogs. Vet. Rec., 91: 571—572.
Garwes, D.J., Lucas, M.H., Higgins, D.A., Pike, B.V. and Cartwright, S.F., 1978/1979. Antigenicity of structural components from porcine transmissible gastroenteritis virus. Vet. Microbiol., 3: 179—190.
Garwes, D.J. and Pocock, D.H., 1975. The polypeptide structure of transmissible gastro-enteritis virus. J. Gen. Virol., 29: 25—34.
Garwes, D.J. and Reynolds, D.J., 1980. The polypeptide structure of canine coronavirus and its relationship to porcine transmissible gastroenteritis virus. J. Gen. Virol. (in press).
Kärber, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Pathol. Pharmakol., 162: 480—483.
Larson, D.J. and Morehouse, L.G., 1976. Transmissible gastroenteritis virus in the neonatal dog. Proceedings International Pig Veterinary Society 1976 Congress, Ames, IA.
McClurkin, A.W., Stark, S.L. and Norman, J.O., 1970. Transmissible gastroenteritis (TGE) of swine: The possible role of dogs in the epizootiology of TGE. Can. J. Comp. Med., 34: 347—349.
Pedersen, N.C., Ward, J. and Mengeling, W.L., 1978. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. Arch. Virol., 58: 45—53.
Pocock, D.H. and Garwes, D.J., 1977. The polypeptides of haemagglutinating encephalo-myelitis virus and isolated subviral particles. J. Gen. Virol., 37: 487—499.
Reynolds, D.J. and Garwes, D.J., 1979. Virus isolation and serum antibody responses after infection of cats with transmissible gastroenteritis virus. Arch. Virol., 60: 161—166.
Reynolds, D.J., Garwes, D.J. and Gaskell, C.J., 1977. Detection of transmissible gastro-enteritis virus neutralizing antibody in cats. Arch. Virol., 55: 77—86.
Witte, K.H., 1971. Micro-color test for assay of transmissible gastroenteritis virus-neutra-lizing antibodies. Arch. Gesamte Virusforsch., 33: 171—176.
Wood, E.N., 1979. Transmissible gastroenteritis and epidemic diarrhoea of pigs. Br. Vet. J., 135: 305—314.