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Detection of Transmissible Gastroenteritis Virus Neutralising Antibody in Cats

By

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With 4 Figures

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Summary

High titres of neutralising activity to transmissible gastroenteritis virus (TGEV), a porcine coronavirus, were found in sera and peritoneal fluids from cats infected with feline infectious peritonitis (FIP). A small proportion of cats, from a hospital population unaffected by FIP, also had neutralising activity. Procedures to remove non-specific viral inhibitors, including treatment by heat inactivation, trypsin, sulphydryl reagent and kaolin absorption were unsuccessful. The active component was unable to neutralise another porcine coronavirus, haemagglutinating encephalomyelitis virus or the porcine enterovirus, Talfan. Gel filtration of feline sera and peritoneal fluid demonstrated high levels of the neutralising activity in the area corresponding to 7 S IgG, which could be removed by absorption with specific anti-IgG serum and these properties are suggested to be consistent with those of antibody. These findings imply that there is a coronavirus in cats which is antigenically related to TGEV and its possible nature is discussed.

Introduction

Transmissible gastroenteritis virus (TGEV) is an established member of the Coronaviridae (17) and infection of pigs with this virus produces specific neutralising antibody in serum. Infection of dogs with the recently described canine coronavirus (1) or TGEV (5, 10) will produce serum antibody which can neutralise TGEV (2). A serological relationship to TGEV has not been established for any other coronavirus. It has recently been published that the agent causing feline infectious peritonitis (FIP) is a coronavirus (6, 12).

This report describes TGEV neutralising activity found in samples of serum and peritoneal fluid from normal and FIP-infected cats.

Materials and Methods

Viruses

The FS 772/70 cloned strain of TGEV; the other porcine coronavirus, haemagglutinating encephalomyelitis virus (HEV), and the porcine enterovirus strain, Talfan were obtained from Miss S. Cartwright and grown in secondary adult pig thyroid (APT/2) cells.

Sera

General Hospital Population

Serum samples were collected at random from cats suffering from surgical or medical conditions, which had been referred to the University of Bristol Veterinary School. A small number of sera from specific-pathogen-free cats, housed in a nearby building were also included. None of the hospitalised animals were known to be suffering from FIP.

FIP Problem Colony

Serum samples were obtained from 16 cats in a commercial colony, where FIP was a disease problem. Sixteen cats had died from FIP in the 3 months prior to sampling. Only animals which had been in contact with previous FIP cases were sampled. Feline peritoneal fluids were obtained by paracentesis from the abdomen of FIP cases, or at post mortem.

Hyperimmune Sera

TGEV hyperimmune serum (PS 713) was produced in a conventional pig by intravenous injection of a preparation of TGE infected piglet intestine, following a method described by Stone *et al.* (16). The TGEV neutralisation titre was 4,500. Serum J 625 was produced by immunisation of a cow with human '0' erythrocytes. The resulting haemagglutination titre for human '0' erythrocytes was 25,600 and was mainly IgM. Monospecific antisera to HEV and Talfan virus were prepared by parenteral injection in specific-pathogen-free pigs.

Neutralisation Tests

Neutralising antibody tests were performed on TGEV and Talfan virus using the microtitre method described for TGEV (18). APT/2 cells or occasionally the continuous APT/92 cell line (14) were used. Results are presented as the reciprocal of the highest dilution of test sample capable of neutralising the virus. End points were calculated by the method of Kärber (8) using triplicate assays for each sample.

Plaque reduction tests were performed to compare neutralising activity for TGEV and HEV. Samples of virus containing 100 PFU and serum dilutions were incubated for one hour at 37° C. Neutralisation of viral infectivity was then measured by plaque assay; TGEV plaques were visualised by staining with 1 per cent neutral red for 4 hours at 37° C. HEV plaques were demonstrated by haemadsorption of rat red blood cells (13).

Haemagglutination Inhibition (HI) Test

Inhibitors of HEV haemagglutination were assayed in serum and peritoneal fluid. Serial dilutions of serum and peritoneal fluid samples were incubated in round-bottomed microtitre plates with four haemagglutinating units of HEV in 0.05 ml for one hour at 37° C. Finally 0.05 ml of a 1 per cent suspension of rat washed erythrocytes was added. The end points were read after 3 to 4 hours at 4° C.

Removal of Non-Specific Inhibitors

Feline sera and peritoneal fluids which possessed neutralising activity for TGEV were treated in three ways known to remove non-specific inhibitors of viruses, that is by heat inactivation (3), kaolin absorption (7) and sulphydryl reagents (9). All serum and peritoneal fluid samples used were heated at 56° C for 30 minutes to inactivate complement. However, as some non-specific viral inhibitors and antibodies are not affected by this treatment, selected sera and peritoneal fluids were also inactivated at 60° C

for 30 minutes, or 65° C for 20 minutes. If trypsin treatment was used in addition to heat treatment, 0.2 ml of 0.25 per cent trypsin, pH 8.5 was added to 0.05 ml of sample. This mixture was then heated at 60° C for 30 minutes before neutralising activity was assayed. Treatment of sera and peritoneal fluids with kaolin was carried out as described by Joo *et al.* (7).

Sera and peritoneal fluids were treated with the sulphydryl reagent 1,4-dithiothreitol (DTT) to destroy macroglobulins. Samples diluted in 50 mM Tris HCl 0.1 M NaCl pH 8.0 were treated with 10 mM or 1 mM DTT at room temperature for one hour. They were then alkylated with iodoacetamide, at a final concentration of 1 mM, for 20 minutes. Overnight dialysis was carried out before assaying for TGEV neutralising activity, or haemagglutination of human '0' erythrocytes.

Assay for Interferon-Like Substances

Samples of sera and peritoneal fluids with detectable levels of TGEV neutralising activity were assayed for interferon-like substances. Suspensions of APT/2 or APT/92 cells were incubated for 24 hours at 37° C with serial dilutions of test samples. The culture fluids were then removed, fresh medium containing viral inoculum added and end points read after 4 days as described for the microtitre neutralisation test.

Ammonium Sulphate Precipitation

Serum and peritoneal fluids were treated with 40 per cent saturated ammonium sulphate for 4 hours at 4° C. The precipitate was deposited by centrifugation at $15{,}000 \times g$ for 20 minutes. Supernatant fluid was withdrawn and the precipitate dissolved in distilled water, to original volume. All samples were then dialysed overnight against 0.85 per cent NaCl and assayed for TGEV neutralising activity as before.

Gel Filtration

The gel filtration system used by Saif *et al.* (15) was followed to determine the approximate molecular size of the neutralising component. Sephadex G-200 (Pharmacia, Uppsala, Sweden) was packed in two columns arranged in series. In the first column (measuring 2.5×45 cm) the direction of flow was descending and in the second (measuring 3.2×85 cm) flow was ascending.

Sample volumes of 3 to 5 ml were applied to the column and eluted with 0.1 M Tris HCl 0.2 M NaCl pH 8.0; 3.0 ml fractions were collected at a flow rate of 8—10 ml per hour and their optical density at 280 nm was measured. Selected fractions were pooled and concentrated by a factor of 25 using Minicon B-15 concentrators (Amicon Ltd., High Wycombe, Bucks) and the product assayed for neutralising activity. Pooled fractions were incubated for 20 hours at room temperature, with monospecific sheep anti-cat IgG serum (this serum had a neutralisation titre for TGEV of less than 2). The precipitate formed was removed by centrifugation at $700 \times g$ for 10 minutes and the supernatant fluid was assayed for neutralising activity. Immunoelectrophoresis of samples before and after adsorption was performed using 1 per cent agarose gels in 0.1 M sodium barbital buffer at pH 8.6.

Results

Occurrence of Neutralising Activity

Table 1 shows neutralising titres for TGEV in cat sera which were randomly obtained from a cat hospital population. A total of 53 sera were tested and 81 per cent were classed as negative, that is the neutralising titre was less than 1 in 10. The majority of the remainder had low positive titres but one was over 256. However, in the cat colony where FIP was a disease problem half of the sera taken were positive and 50 per cent of these had TGEV neutralising titres of more than 1 in 50.

Peritoneal fluid was taken from 12 cats in the problem colony, all of which had histologically confirmed lesions of FIP. Table 2 shows that 11 out of 12 had a positive TGEV neutralising test. Negative results were obtained with peritoneal fluids from two colony cats which had no lesions of FIP at post-mortem and also three peritoneal fluids obtained from normal cats in the general population and one from an FIP case which occurred outside the colony. Serum, pleural and peritoneal fluids from one FIP case had comparable titres as shown in Table 3. Likewise coagulated and heparinised samples gave similar results.

Table 1. *Incidence of TGEV neutralising activity in cat sera obtained from a hospital population or an FIP problem colony*

	Total number	TGEV neutralisation titre				
		<10	10—50	50—100	100—256	>256
General hospital population	53	43	9	—	—	1
Problem colony	16	8	4	1	—	3

Table 2. *Incidence of TGEV neutralising activity in feline peritoneal fluids obtained from cases of feline infectious peritonitis (FIP) or normal cats in a general hospital population or from an FIP problem colony*

	FIP	TGEV neutralisation titre				
		<10	10—50	50—100	100—256	>256
General hospital	+	1	—	—	—	—
population	—	3	—	—	—	—
Problem colony	+	1	1	2	3	5
	—	2	—	—	—	—

Table 3. *TGEV neutralising titres in serum, pleural or peritoneal fluid from an FIP case*

Sample[a]	TGEV neutralisation titre
Serum 1	1,430
2	2,270
Pleural fluid 3	1,430
Peritoneal fluid 4	1,140

[a] Serum sample 1 was taken from a cat with clinical FIP. Samples 2, 3 and 4 were obtained from the case post-mortem, one week later

Specificity of Neutralising Activity

The specificity of the TGEV neutralising activity in feline sera and peritoneal fluids was first investigated by testing them with HEV and Talfan virus. As shown in Table 4, all the samples tested failed to neutralise either virus. They did inhibit haemagglutination by HEV but this was eliminated by absorption

with kaolin. Control antisera to TGEV, HEV and Talfan virus failed to neutralise the heterologous viruses.

Table 4. *Viral neutralisation and haemagglutination titres of feline peritoneal fluids from cats with feline infectious peritonitis and specific antisera to the viruses tested*

Sample	Viral neutralisation titres			HEV HI	
	TGEV	Talfan virus	HEV	Untreated	Kaolin adsorbed
Feline peritoneal fluid 61	3,600	<2	<8	256	<16
Feline peritoneal fluid 65	2,276	<2	<8	512	<16
Feline peritoneal fluid 67	11,462	<2	<8	1,024	<16
TGEV antiserum PS 713	4,500	<2	<8	16	< 8
Talfan antiserum	<2	>2,560	<8	160	<80
HEV antiserum	<2	<2	>10,240	512	256

Removal of Non-Specific Inhibitors

The heat stability of the neutralising factor was tested and compared with TGEV hyperimmune porcine antiserum (PS 713). Table 5 shows some reduction in titre at 60° C for PS 713 and one peritoneal fluid but no more at 65° C for PS 713, feline serum or peritoneal fluids. Trypsin treatment had little further significant effect on samples than heat inactivation at 60° C alone.

Table 5. *Effect of heat inactivation at 56°, 60° and 65° C and trypsin treatment on feline serum and peritoneal fluids*

Sample	TGEV neutralisation titre after heating at:			
	56° C	60° C	65° C	60° C + Trypsin
TGEV antiserum PS 713	4,500	2,270	2,270	2,270
Feline peritoneal fluid 61	3,600	1,196	1,196	450
Feline peritoneal fluid 62	89	NT[a]	88	NT
Feline peritoneal fluid 87	2,270	3,607	NT	1,135
Feline serum 87	1,790	1,808	NT	904

The results of kaolin treatment of sera was unpredictable as at times immunoglobulin was removed as well as non-specific inhibitors. For example, the treatment of undiluted PS 713 reduced the antibody titre from 4,500 to 573 (Table 6) and of diluted PS 713 reduced the antibody titre to below 80. The effect of kaolin adsorption on the neutralising titre of peritoneal fluid 61 was less than that of PS 713. Table 4 shows that kaolin was completely effective in removing inhibitors of HEV haemagglutination.

The results of treating PS 713 and two of the feline peritoneal fluids with DTT are also shown in Table 6. Within a range of one doubling dilution, the three

samples were unaffected by either concentration of DTT. The haemagglutination titre of bovine serum J625 was reduced 50 fold by 1 mM DTT.

All samples were assayed for interferon-like activity, by pretreatment of both APT/2 cells and the APT/92 cell line; no activity was found.

Table 6. *Effect of kaolin adsorption and DTT on feline peritoneal fluids*

	TGEV neutralisation titre				
		Kaolin adsorbed[a]		DTT treated	
Sample	Untreated	Undiluted	1/10	10 mM	1 mM
TGEV antiserum PS 713	4,500	572	<80	2,270	2,276
Feline peritoneal fluid 61	1,851	NT[b]	903	2,522	2,001
Feline peritoneal fluid 65	4,552	NT	NT	2,270	3,607
	Haemagglutination titre for human '0' erythrocytes				
Bovine serum J 625	25,600	NT	NT	NT	500

[a] Samples were treated with 3 volumes of kaolin (25 per cent wt/wt) undiluted or after dilution (1 in 10). Data refer to the neutralising activity extrapolated to undiluted sample

[b] NT Not tested

Ammonium Sulphate Precipitation

The neutralising factor in feline samples was completely precipitated with 40 per cent saturated ammonium sulphate. Furthermore, centrifugation of untreated samples under the conditions used to collect the ammonium sulphate precipitate did not alter the neutralising titre. In addition no activity was detected in the concentrated dialysate.

Gel Filtration

Gel filtration produced three peaks of protein (Figs. 1, 2, 3) the first after the void volume containing predominantly macroglobulins including IgM. The second peak contained 7S IgG, whilst the third peak represented smaller molecular

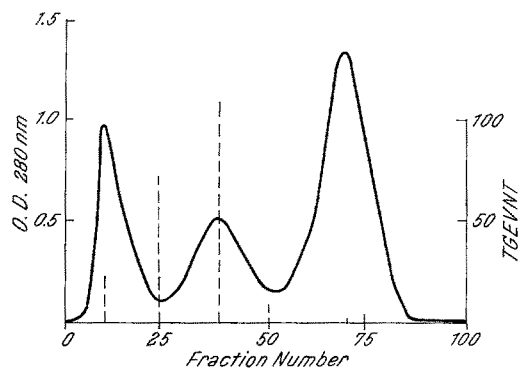

Fig. 1. Sephadex G-200 elution profile of PS713. Fractions were concentrated by a factor of only 10 before assay for neutralising antibody
——— optical density (O.D.) at 280 nm; — — — TGEV neutralisation titre (NT)

weight serum components, including albumin. The profile of TGEV hyperimmune antiserum (PS 713) is shown in Figure 1. Maximum neutralising antibody concentration was found to coincide with the second protein peak. Lower level activity was found in the first peak and in the trough between the first and second peaks. These higher molecular weight neutralising components probably correspond to 7S IgG complexes, IgA or IgM.

Feline serum and peritoneal fluid from FIP infected cats showed similar profiles. The optical density of the second peak was extremely high in FIP cases as a result of hypergammaglobulinaemia, a characteristic of the disease. This peak contained high levels of neutralising activity in both serum and peritoneal fluids as shown in Figures 2 and 3. Very little activity was found in the first peak region and it was not affected by DTT. No activity was found in the third peak. Pooled fractions from the second peaks of both feline serum and peritoneal

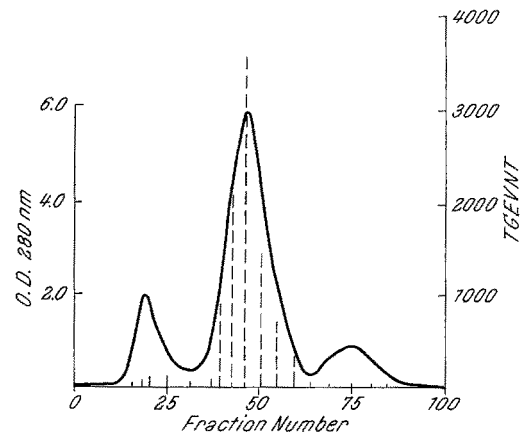

Fig. 2. Sephadex G-200 elution profile of feline serum from an FIP case. Fractions were concentrated 25 times before assay for TGEV NT
——— O.D. at 280 nm; — — — TGEV NT

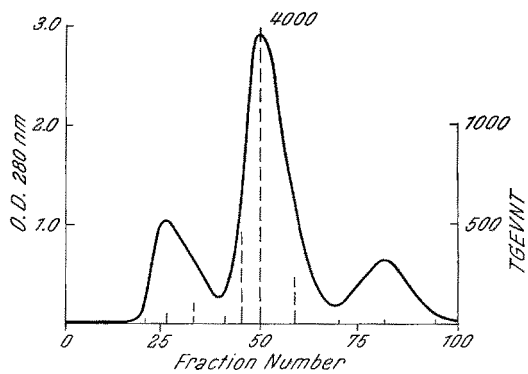

Fig. 3. Sephadex G-200 elution profile of feline peritoneal fluid from an FIP case. Fractions were concentrated 25 times before assay for TGEV NT
——— O.D. at 280 nm; — — — TGEV NT

fluid were adsorbed with sheep anti-cat IgG. Before adsorption immunoelectrophoresis of both samples (Fig. 4) showed a single precipitin arc of IgG, using sheep anti-cat IgG as precipitating antiserum. TGEV neutralising titres of 2,825 for feline serum and 710 for peritoneal fluid were recorded. However, after adsorption both titres were reduced to less than 20 and the IgG precipitin lines were lost completely.

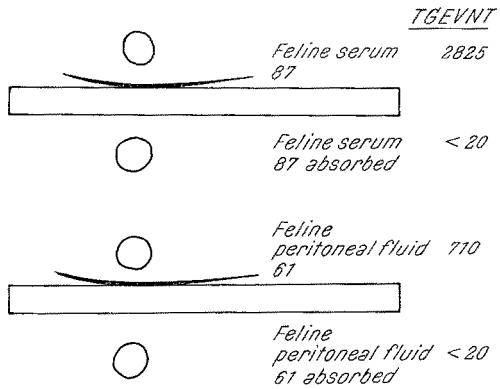

Fig. 4. Immunoelectrophoresis of feline serum (87) and peritoneal fluid (61). Concentrated pooled fractions of the second peak from G-200 were used, before and after adsorption with monospecific sheep anti-cat IgG

Discussion

This report shows that sera and peritoneal fluids from cats affected with FIP neutralise TGEV *in vitro*. The active component was not destroyed by heating at 56°, 60° or 65° C and trypsin treatment had little effect. DTT did not destroy the TGEV neutralising activity, although it was shown to be fully effective under the conditions used, by the effect on the IgM in the bovine serum tested. Thus the activity demonstrated in feline fluids was unlikely to be a macroglobulin. Feline sera and peritoneal fluids were not more affected by kaolin absorption than specific antibody. Most non-specific inhibitors, such as that described for avian infectious bronchitis virus (9), are affected by one or more of these methods. As pretreatment of cells was not effective it is unlikely that the activity was mediated by interferon-like substances. The range of action of the neutralising activity did not include HEV, which is antigenically dissimilar to TGEV and is not related by cross-neutralisation. Talfan virus, a porcine enterovirus, was not affected.

Gel filtration studies demonstrated that the activity predominated in the gamma globulin fraction. The highest neutralising titre was found in the area corresponding to 7S IgG and indicates that the neutralising activity may well be antibody; in addition, specific removal of IgG by adsorption, which was confirmed by immunoelectrophoresis, completely removed the neutralising activity for TGEV. The patterns of neutralising activity in Figures 2 and 3 correspond well to that shown for an inhibitor in bovine serum for respiratory syncytial virus (3). This inhibition was shown subsequently to be antibody when bovine respiratory syncytial virus was isolated as the corresponding antigen.

The high levels of gamma globulin in sera and peritoneal fluids from FIP cases are commonly found associated with this disease (4). Assuming that the TGEV neutralising activity in feline sera is due to specific immunoglobulin, the data presented do not demonstrate whether the high levels found in FIP cases are due to generalised stimulation of gamma globulin production or to a specific immune response to a related antigen associated with FIP.

A virus associated with FIP is the only feline coronavirus described in the literature. It may be serologically related to TGEV as is the canine enteric coronavirus. Recent evidence (12) suggests that antibody to FIP is more widely spread in the feline population than the clinical disease itself. Thus the occurrence of anti-TGEV activity in apparently uninfected cats does not rule out an association with FIP. Definite evidence of this association is yet to be established. There could be a separate coronavirus, not isolated, responsible for this activity and a further investigation of this possibility is in progress.

The finding that some cats have antibodies capable of neutralising TGEV is of interest in epidemiological studies of TGEV in pigs. It indicates that serum neutralising titres to TGEV cannot be used to demonstrate whether cats could act as carriers of the disease. The same problem has been encountered in studies on the role of dogs in the spread of TGEV. Comparative work on the nature of the coronaviral antigens and the serum antibody response to them in various species, is currently in progress.

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