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Antigenic Relationships Among Homologous Structural Polypeptides of Porcine, Feline, and Canine Coronaviruses

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Transmissible gastroenteritis virus of swine (TGEV), feline infectious peritonitis virus (FIPV), and canine coronavirus were studied with respect to their serological cross-reactivity in homologous and heterologous virus neutralization, immune precipitation of radiolabeled TGEV, electroblotting, and enzyme-linked immunosorbent assay using individual virion polypeptides prepared by polyacrylamide gel electrophoresis. TGEV was neutralized by feline anti-FIPV serum, and the reaction was potentiated by complement; heterologous neutralization involved antibody reacting with the peplomer protein (P), the envelope protein (E), and cellular (glycolipid) components incorporated into the TGEV membrane. Electrophoretic analysis of immune precipitates containing [^{35}S]methionine-labeled disrupted TGEV and feline anti-FIPV antibody confirmed the reaction with the P and E polypeptides and showed the nucleocapsid protein (N) in addition. Electroblotting, followed by incubation with antibody, ^{125}I-labeled protein A, and fluorography, disclosed cross-reactions between the three viruses at the N and E levels and revealed differences in the apparent molecular weights of the latter. Enzyme immunoassays performed with standard amounts of immobilized P, N, and E polypeptides of the three viruses showed recognition of the antigens by homologous and heterologous antibody to comparable degrees. These results indicate a close antigenic relationship between TGEV, FIPV, and canine coronavirus due to common determinants on the three major virion proteins. The taxonomic implications of these findings are discussed.

Coronaviruses are enveloped RNA viruses causing respiratory, enteric, and generalized disease in mammals and birds. Virions are roughly spherical, measuring about 120 nm in diameter and possessing widely spaced, club-shaped projections (peplomers). The morphological criteria for classification have been supplemented by recent data on the chemical composition of the virus particle and the transcription strategy of its genome (Siddell et al., in press).

The inter- and intraspecies serological relationships within the Coronaviridae family are poorly understood. During the last years, however, indications for the existence of antigenic clusters have been obtained. One of these was based on the observation of heterotypic reactions between transmissible gastroenteritis virus of swine (TGEV) and feline infectious peritonitis virus (FIPV) by Witte et al. (22); these findings were confirmed and subsequently extended to include an enteric canine coronavirus (CCV) and the respiratory isolate 229E from humans (1, 17, 20).

Infection of pigs with TGEV results in a gastroenteritis that is most severe, and frequently fatal, in piglets younger than 3 weeks of age. Upon postmortem examination, necrosis of the villous epithelium, with subsequent atrophy in the jejunum and the ileum, is prominent (16, 23). Feline infectious peritonitis (FIP) is a progressively debilitating, usually fatal, immune-mediated condition affecting domestic and wild Felidae and is characterized by diffuse fibrinous polyserositis, mesothelial hyperplasia, and focal necrosis in the parenchymatous organs. The disease is a rare exception, and seroconversion is the only sign of infection with FIPV in most cases (9). CCV was isolated in 1971 from fecal specimens of dogs suffering from gastrointestinal disease (1); the agent is widespread in the canine population, and most infections seem to take an inapparent course.

The aim of the present study was to investigate the nature and degree of the antigenic relatedness between TGEV, FIPV, and CCV at

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the level of the individual viral polypeptides. The results may have implications for classification (e.g., establishment of genera) within the Coronaviridae family.

MATERIALS AND METHODS

Cells and virus. The swine kidney cell line PD5 (kindly provided by Philips Duphar, Weesp, The Netherlands) and the fcwf line of feline whole fetus cells (Pedersen et al., unpublished data) were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). The Purdue and Miller 3 strains of TGEV (gifts by M. Pensaert, Ghent, Belgium, and R. D. Woods, National Animal Diseases Laboratory, Ames, Iowa, respectively) were used for infection of confluent pig cell monolayers at a multiplicity of infection of ≥10. After 1 h at 37°C, the inoculum was removed, and DMEM supplemented with 2% FCS was added. The cultures were incubated further and harvested between 15 and 20 h after infection before the appearance of extended cytopathic effect. Infectivity titers of about 10^9 50% infective doses (ID_{50})/ml were usually obtained in the supernatants of roller cultures. For use in neutralization experiments, the Purdue strain of TGEV had been adapted to growth in feline cells; after 10 passages in Crandell feline kidney cells, titers of $>10^6$ ID_{50}/ml were routinely obtained.

Both the feline coronavirus and the CCV were field isolates from the University of California, Davis; after five passages they reproducibly reached titers of 10^4 and 10^6 ID_{50}/ml, respectively, in fcwf cell cultures. Virus harvests obtained by three cycles of freezing and thawing were stored at −70°C until use.

Infectivity titrations were performed by adding suspensions of the respective cells (2×10^4/50 μl) to serial 10-fold dilutions of virus material in DMEM (180 μl per well of a flat-bottom microtiter plate). Virus had been diluted in six replicate titrations using a 20-μl multichannel pipette (Titertek; Flow Laboratories, Inc., Glasgow, Great Britain). Cytopathic effect was read microscopically; titers were calculated using the Kärber formula and expressed as ID_{50}/ml.

Purification of virus. Fresh harvests from roller cultures (TGEV) or frozen samples were clarified by low-speed centrifugation; the virus was precipitated by the dropwise addition of 40% (vol/vol) of a saturated solution of ammonium sulfate. After 6 to 18 h of incubation at 4°C, the virus suspension was centrifuged at 15,000 × *g* for 20 min. The pellet was resuspended in $^1/_{10}$ to $^1/_{40}$ of the original volume of TES buffer (0.02 M Tris-hydrochloride, 1 mM EDTA, 0.1 M NaCl, [pH 7.4]) and clarified (5 min, 10,000 × *g*). This material was layered onto 10% (wt/wt) sucrose in TES buffer on top of a 60% (wt/wt) sucrose cushion in tubes of a Spinco SW27.0 rotor. After centrifugation for 3 h at 25,000 rpm, the light-scattering band at the sucrose interface was collected and diluted in Tris-hydrochloride buffer (0.02 M, pH 8.0). At this stage, the total protein concentration of the sample was about 0.3% of the starting material; it was of sufficient purity for use in preparative electrophoresis and electroblotting (see below). Labeled TGEV for radioimmune precipitation was further purified by rate-zonal centrifugation in a linear 20 to 45% sucrose gradient spun for 3 h at 25,000 rpm in a Spinco SW27 rotor. The procedure is essentially the same as that described by Garwes and Pocock (4).

Antisera and neutralization assay. Porcine anti-TGEV hyperimmune, convalescent, and antibody-free sera from the Netherlands, Belgium, Germany, and the United States were used. Anti-FIPV preparations were ascites fluids from fatal cases or sera collected from field cases at different stages of the infection; control sera were obtained from a specified pathogen-free (SPF) cattery (CPB-TNO Zeist, The Netherlands). Convalescent sera from dogs experimentally infected with CCV were employed; an SPF canine serum was a gift from Antibodies Inc., Davis, Calif. Rabbit anti-swine, anti-cat, and anti-dog immunoglobulin G (IgG) conjugates (heavy and light chains) to horseradish peroxidase were obtained from Cappel Laboratories, Cochranville, Pa.; unlabeled anti-species IgG sera were purchased from Miles-Yeda Ltd., Rehovot, Israel.

For microtiter neutralization assays, 100-μl volumes of serial 10-fold TGEV dilutions in DMEM containing 10% heat-inactivated (30 min at 56°C) FCS and 2 μg of RNase A per ml (Boehringer Mannheim Corp., Germany) were mixed with 50 μl of an appropriate dilution of heat-inactivated serum or ascitic fluid. After the addition of 50 μl of fresh or heat-inactivated SPF pig or cat serum diluted 1:10, the mixtures were incubated at 37°C in a humid 5% CO_2 atmosphere. After 1 h, the cell suspension (4×10^5 cells per ml) was dispensed in 50-μl volumes, and the plates were incubated for 3 to 5 days before being read by microscopy.

Radioimmune precipitation and PAGE. PD5 cells grown in stationary monolayer cultures were infected with TGEV as described. Three hours after the addition of the DMEM-1% FCS, the medium was removed, the cells were rinsed three times with phosphate-buffered saline, and labeling medium was added; it consisted of Eagle minimal essential medium with reduced methionine (25 nmol/liter) supplemented with 1% dialyzed heat-inactivated FCS, containing 1 μg of actinomycin D per ml and 10 nmol of [^{35}S]methionine per liter (specific activity, 800 Ci/nmol; Radiochemical Centre, Amersham, England). Virus was harvested 24 h after infection and purified as described above; trichloroacetic acid-precipitable radioactivity was determined with a liquid scintillation spectrometer using a toluene scintillant. Labeled TGEV (100 μl) was disrupted by the addition of 50 μl of TES buffer containing 1.5% Triton X-100 and 1.5% 1,5-naphthalenedisulfonate · Na_2 and reacted with 25 μl of a 1:10 serum dilution for 18 h at 4°C. The sera had been centrifuged at 10,000 × *g* for 15 min before use. Precipitation of the immune complexes was achieved after the addition of 30 μl of 3.0 M KCl and 50 μl of an undiluted rabbit anti-species IgG serum and overnight incubation at 4°C. The precipitate was collected by centrifugation at 10,000 × *g* for 5 min, washed three times with TES buffer, and prepared for polyacrylamide gel electrophoresis (PAGE) by the addition of 25 μl of sample buffer and heating to 95°C for 3 min. The sample buffer consisted of 0.0625 M Tris-hydrochloride, pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, and 0.001% bromphenol blue. Electrophoresis was performed in slab gels (10 cm long and 1.5 mm thick) containing 12.5% acrylamide, 0.1% bisacrylamide, 375

TABLE 1. Survival of TGEV adapted to porcine and feline cells in homologous and heterologous neutralization tests and the influence of complement[a]

Source of complement	Source of antibody	Prepn no.	% Survival of: TGEV porcine[b] Plus complement	TGEV porcine[b] Plus inactivated complement[c]	TGEV feline[b] Plus complement	TGEV feline[b] Plus inactivated complement[c]
Pig	Anti-TGEV hyperimmune sera (1:200)	II	0.01	0.02 (2)[d]	0.01	0.01 (1)
		X	3.2	3.2 (1)	4.7	2.2 (<1)
		XII	0.01	0.02 (2)	0.1	0.2 (2)
	Ascites fluids from FIP cats (1:20)	9	7	100 (15)	1	47 (47)
		35	0.7	32 (45)	0.03	100 (3,000)
		36	10	46 (5)	0.7	15 (21)
Cat	Ascites fluids from FIP cats (1:20)	9	35	15 (<1)	5	32 (7)
		35	10	32 (3)	0.3	32 (105)
		36	3	21 (7)	ND[e]	ND —

[a] The ID_{50} of every mixture in the presence of heat-inactivated SPF pig and cat serum was taken as 100%.
[b] Titrations were made in porcine and feline cells.
[c] The serum serving as the complement source had been inactivated for 30 min at 56°C in these experiments.
[d] Numbers in parentheses are the ratios of virus survival in the presence of heated pig or cat serum to that in unheated pig or cat serum.
[e] Not determined.

mM Tris-hydrochloride (pH 8.8), and 0.1% SDS. A 1-cm-long stacking gel was used, containing 4% acrylamide, 0.1% bisacrylamide, 125 mM Tris-hydrochloride (pH 6.8), and 0.1% SDS. The reservoir buffer contained 50 mM Tris base, 384 mM glycine, and 0.1% SDS. Electrophoresis was at 100 V for about 5 h. Radioactive polypeptides were detected by fluorography (11). For quantitative estimates of precipitation of the individual polypeptides, the respective regions were cut out from the dried gel and counted in a Triton X-100–toluene scintillant.

Electroblotting of unlabeled proteins. Since glycine from Laemmli gels interferes with the covalent binding of proteins to diazophenylthioether (DPT) paper, we have used the 13/1 Tris-borate system for PAGE as described by Neville (15). The separated proteins were transferred electrophoretically to DPT paper; diazotization had to be timed with the end of the electrophoretic run. Aminothiophenol paper had been prepared using a procedure developed by Brian Seed, Division of Biology, California Institute of Technology, Pasadena; for diazotization it was immersed into an ice-cold solution containing 100 ml of 1.2 N HCl and 2.7 ml of 0.1% (wt/vol) $NaNO_2$. After 20 to 40 min at 0°C, the paper was washed in water and subsequently in transfer buffer (sodium phosphate buffer, 15 mM, pH 6.5). At that time, the gel had also been washed three times with transfer buffer; it was covered free of air bubbles with the activated paper and placed into an electrophoresis device (Electroblot; EC Apparatus Corp., St. Petersburg, Fla.). Transfer was allowed to proceed at 37°C for 1 h at 3 to 4 A and 40 V; thereafter, remaining diazo groups were blocked with 0.25% gelatin in a 0.1 M Tris-hydrochloride buffer, pH 9.0. For visualization of the transferred proteins, the paper was first incubated in antiserum diluted 1:100 in incubation buffer (TES buffer containing 0.05 Nonidet P-40 and 0.25% gelatin); after 2 h of rotation at room temperature, the paper was washed three times for 1 min in incubation buffer and subsequently incubated with 5 to 10 μCi of ^{125}I-labeled protein A per cm^2 for 1 h. All incubations were made in Seal-a-meal FDA bags (Dazey Products, Kansas). Autoradiography was performed on prefogged Kodak XR film at −70°C (12).

Several sera were used with the same antigen preparation. To remove antibody from the paper, it was washed twice for 1 h at room temperature in a 50 mM Tris-hydrochloride buffer, pH 8.0, containing 8 M urea and 2% 2-mercaptoethanol. After equilibration in incubation buffer, another serum could be tested.

Gel electrophoresis-derived ELISA. After SDS-PAGE of an unlabeled virus preparation, the gel slab was fractionated into horizontal 5-mm strips (perpendicular to the direction of electrophoresis). The strips were soaked in water (three times for 5 min) to remove excess SDS and subsequently Dounce homogenized in 3.0-ml volumes of Tris-hydrochloride buffer (0.02 M, pH 8.0) containing 0.02% NaN_3. The gel slurries were left at +4°C for elution. The protein concentrations of the eluates were calculated from the absorbance curve of Coomassie blue-stained gel strips cut vertically at both sides from the slab before fractionation.

Flat-bottom Microelisa polystyrene plates (Dynatech Laboratories, Billingshurst, Sussex, Great Britain) were coated with viral protein in 100 μl of 0.1 M Na_2CO_3, pH 9.6, per cup at a standard concentration of 500 ng per well. After incubation at 37°C for 3 h, the plates were washed three times using a 0.15 M NaCl–0.1% Tween 20 solution. Antiserum dilutions were made in a buffer containing 0.05 M Tris-hydrochloride (pH 7.4), 0.15 M NaCl, 1 mM disodium EDTA, 0.1% bovine serum albumin, 0.05% Tween 20, and 0.02% NaN_3. They were dispensed into the wells in 100-μl volumes and incubated at 37°C for 2 h. After another three cycles of washing, the anti-IgG peroxidase conjugate (100 μl) was added at a dilution of 1:400, and incubation was continued for 1 h. Finally, the substrate solution (300 μl per well) was pipetted into the wells; it consisted of 25 μl of a solution of 2,2′-azino-di(3-ethyl-benzthiazoline sulfonic acid) diammonium

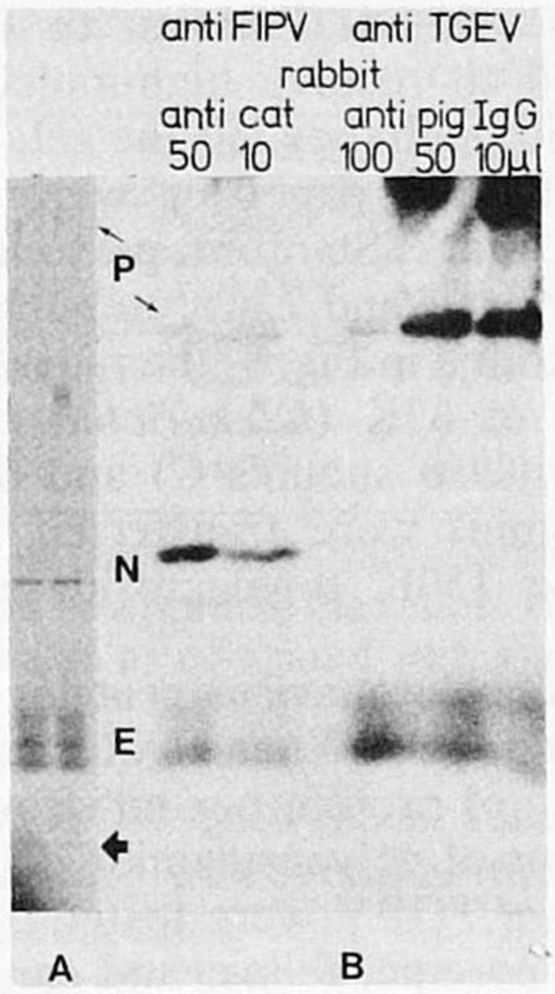

FIG. 1. SDS-PAGE of [^{35}S]methionine-labeled TGEV. (A) Material from sucrose gradient fractions shows the peplomer (P), the nucleocapsid (N), and the envelope (E) polypeptides; an additional minor protein (heavy arrow) was resolved. (B) Immune precipitation using homologous (anti-TGEV) and heterologous (anti-FIPV) antibody resulted in demonstration of the three major polypeptides by the latter; note the absence of N protein in the homologous reaction and accumulation of radioactivity at the stacking gel interface. Normal and SPF pig serum did not result in precipitation of significant radioactivity (not shown).

salt (100 ml/4.5 ml of water, ABTS; Sigma Chemicals Co., St. Louis, Mo.) and 20 μl of a 2% solution of H_2O_2 diluted in 5.0 ml of a 50 mM solution of citric acid. After 5 to 20 min at room temperature, the reactions were read in a Titertek Multiscan Reader (Flow Laboratories, Inc.) at a wavelength of 405 nm in the enzyme-linked immunosorbent assay (ELISA) (2).

RESULTS

Heterologous neutralization. Of 59 heat-inactivated ascites fluids from field cases of FIP tested at a dilution of 1:10, 11 showed distinct neutralization of TGEV infectivity in PD5 cells; in the presence of unheated pig serum the number increased to 29. Significant potentiation of heterologous neutralization was achieved when the reaction mixture was supplemented with fresh serum; this measure had no effect on TGEV neutralization by homologous serum (Table 1). When TGEV adapted to growth in feline cells was assayed in neutralization experiments, unheated pig serum again had no effect on the homologous reaction, whereas with feline ascitic fluids, enhancement was even more pronounced as compared with neutralization of TGEV grown in pig cells. Unheated cat serum did also potentiate neutralization in both cell systems, although to a lesser extent. It should be noted that in our hands, normal pig and cat sera alone (diluted 1:10) reduced TGEV infectivity by 32 to 85%, depending upon the host cell species. The data in Table 1 have been corrected for these decrements.

Acetic acid-ethanol (1:3)-fixed PD5 cell monolayers or unfixed preparations were used for absorption of an ascitic fluid before titration of neutralizing activity against 20 ID_{50} of pig cell-grown TGEV. The titer in the presence of fresh pig serum of the unabsorbed ascitic fluid was 200. After the first and second absorptions, it had dropped to 100 and 70, respectively; three additional absorptions showed no further decrease in neutralizing activity.

Radioimmune precipitation. The structural polypeptides of TGEV were resolved on 12.5% SDS-polyacrylamide gels (Fig. 1A). The peplomer (P) protein (160,000 ± 16,000 [160 ± 16K], n = 6), the nucleocapsid (N) proteins (56 ± 6K, n = 4), the heterogeneous envelope (E) protein (33 ± 2K to 26 ± 1K, n = 7), and an additional minor polypeptide (21 ± 2K, n = 3) were found in virus from sucrose gradient fractions containing the peak of infectivity. The three major polypeptides (4) were also recovered in immune precipitates using ascitic fluid from a FIP case; homologous porcine antiserum, however, did not reveal the N protein in the electropherogram. In none of nine anti-TGEV immune and hyperimmune pig sera tested subsequently (results not shown) did the N protein appear; however, significant amounts of label invariably remained at the top of the stacking gel (Fig. 1B). In contrast, immune precipitates obtained with different sera from FIP cats could be resolved into the three major TGEV proteins. Between 80

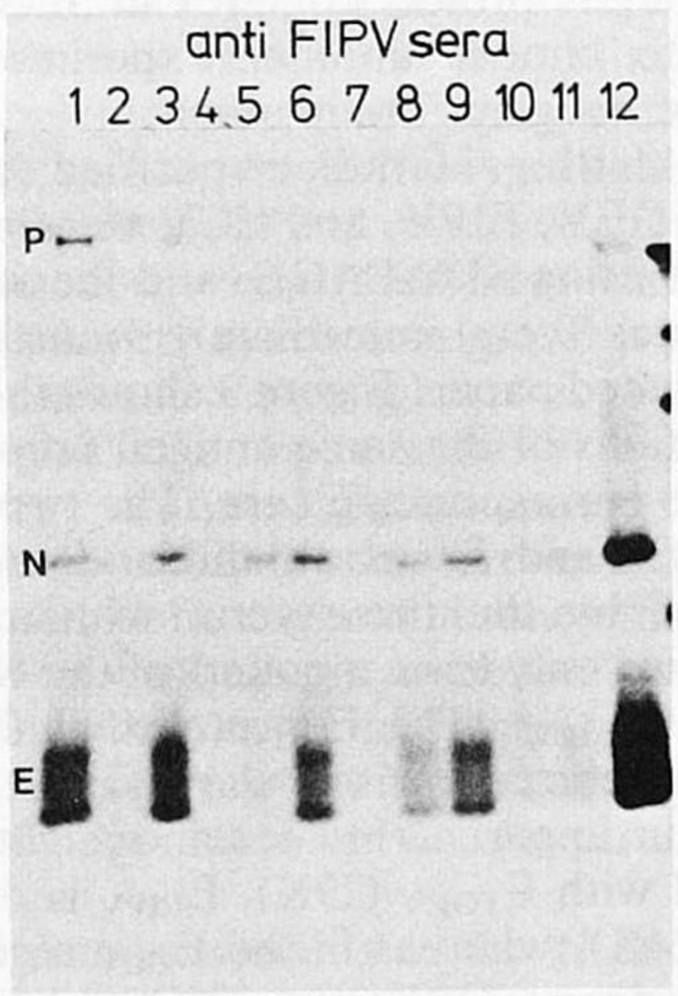

FIG. 2. SDS-PAGE of [^{35}S]methionine-labeled TGEV after immune precipitation using 12 different sera from FIP field cases at different stages of the infection. Note the poor recognition of the peplomers (P) and the prominent reaction with the envelope (E) polypeptide.

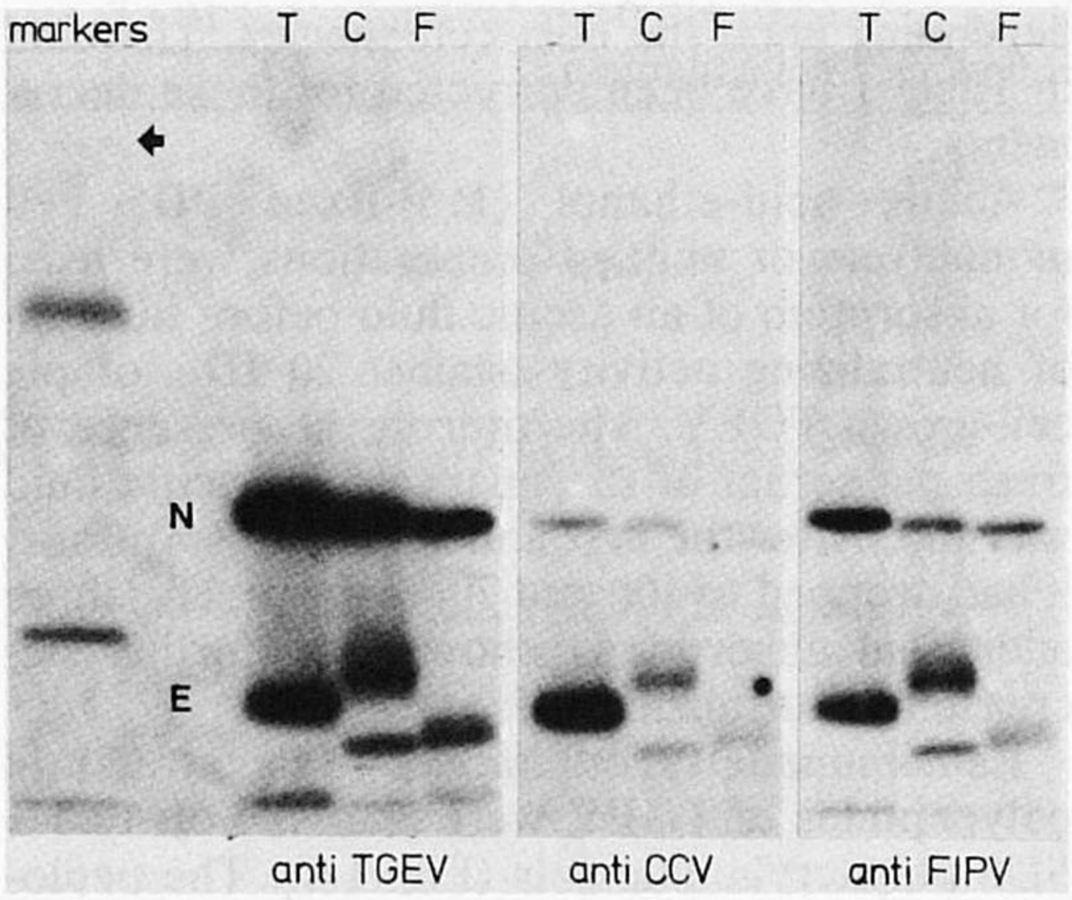

FIG. 3. Autoradiographs from the same SDS-polyacrylamide gel electroblot after incubation with antiserum against TGEV (left), CCV (middle), and FIPV (right), followed by ^{125}I-labeled protein A. Purified TGEV (left lanes), CCV (middle lanes), and FIPV (right lanes) preparations were electrophoresed in parallel, together with three iodinated marker proteins (33K, 67K, and 130K), and subsequently transferred to DPT paper. Note the absence of the largest marker (position indicated by arrow) and the peplomer protein which should appear near the upper margin of the figure.

and 99% of the total radioactivity per precipitate was found in the region of the E protein, less than 1 to 17% in the N region, and 2% or less in the P region of the gel (Fig. 2). Purified [^{35}S]methionine-labeled TGEV alone showed the following distribution of activity: E, 85%; N, 5%; P, 10%. Due to the aberrant behavior of the pig antibody-N protein complex in SDS-PAGE, we had to choose another experimental approach for antigenic comparison.

Electroblotting. Gradient-purified preparations of TGEV, FIPV, and CCV were analyzed in parallel using SDS-PAGE, and the separated polypeptides were immediately transferred to DPT-activated paper. Figure 3 shows the results of incubations of the same antigen preparations with three corresponding sera. The two smaller proteins (N and E) of all three viruses were recognized by the three sera, with anti-CCV precipitating only trace amounts of the N protein of FIPV (N_{FIPV}). The E proteins of the three viruses under comparison showed distinct differences in apparent molecular weight. When compared with E_{TGEV} (29K), E_{FIPV} is distinctly smaller (24K), whereas in the E_{CCV} region, two separate species (32K and 22K) are resolved. Quantitative conclusions cannot be drawn from these experiments; it is obvious, however, that the anti-CCV serum used contained little anti-N activity.

The P proteins are not visualized in the autoradiographs. This is inherent to the method, which failed to resolve high-molecular-weight proteins. Although they left the gel, they did not bind to activated paper or were lost during washing (G. Stark, Stanford, personal communication; Horzinek and Lutz, unpublished data). As demonstrated in Fig. 3, the radioactive marker proteins of 33K (*Escherichia coli* aspartic transcarbamylase subunit C) and 67K (bovine serum albumin) were transferred and bound, whereas the 130K β-galactosidase marker is missing.

ELISA using coronavirus proteins eluted from polyacrylamide gels. When eluates (calculated to contain 5 μg of protein per ml) from the P, N, and E regions of polyacrylamide gels after electrophoresis of TGEV were tested against serial dilutions of porcine, feline, and canine immune sera, reaction patterns as shown in Fig. 4 were obtained; homologous serum showed the strongest reaction, anti-FIPV serum an intermediate

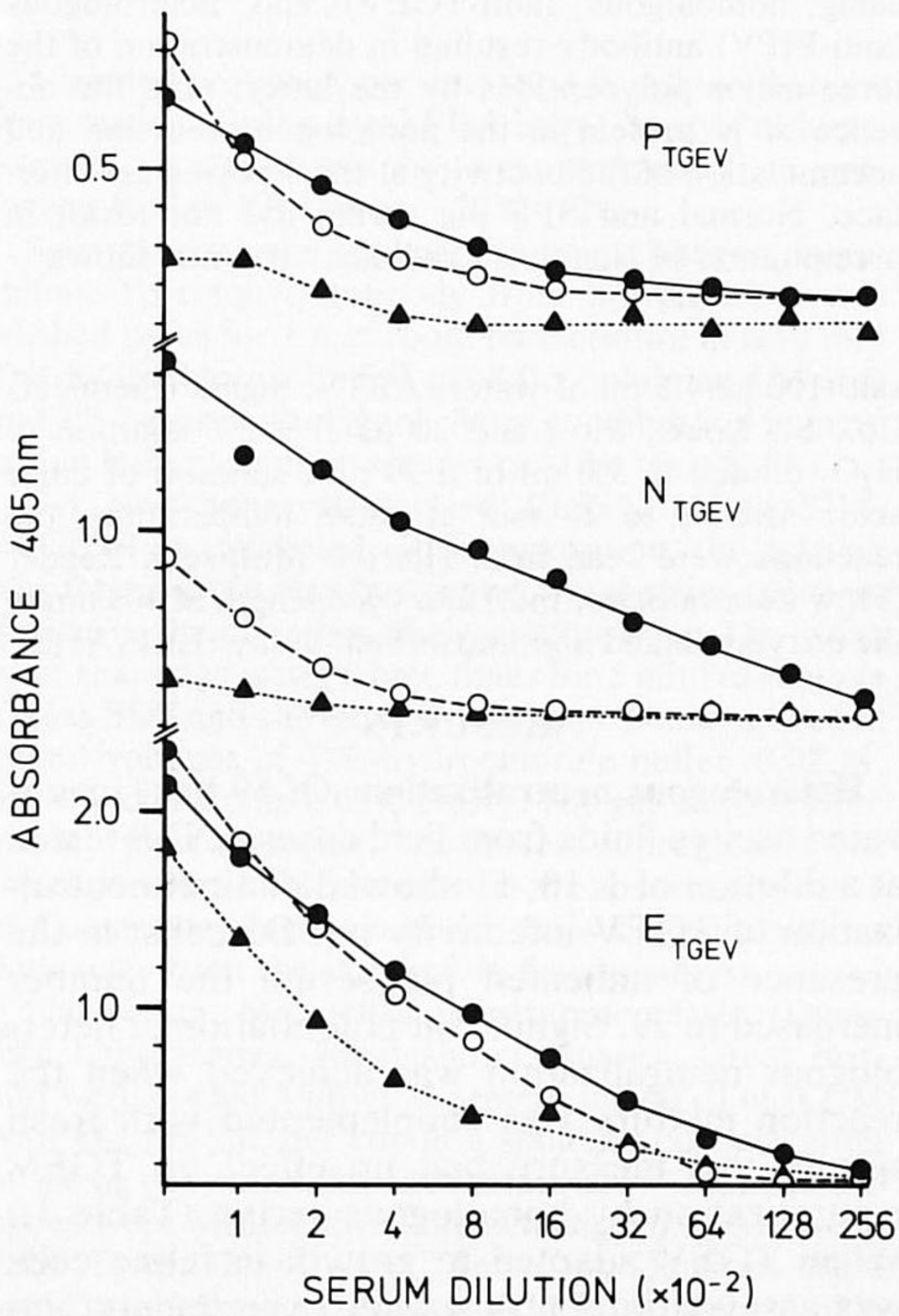

FIG. 4. ELISA using a standard antigen concentration (5 μg/ml) of the P, N, and E proteins of TGEV coupled to a polystyrene solid phase. The reaction of homologous (pig anti-TGEV serum, ●) and heterologous antibody (cat anti-FIPV serum, ○; dog anti-CCV serum, ▲) is shown after incubation with the respective anti-species IgG–horseradish peroxidase conjugates. Sera from SPF pigs, cats, and dogs gave baseline values in this experiment (not shown). Note the different scale units for the peplomer protein.

TABLE 2. Ratios of ELISA values of absorbance at 405 nm obtained in tests using a standard antigen concentration and one antiserum dilution[a]

Virus	Ratios of ELISA values of absorbance at 405 nm for the following antigens and antisera:								
	Peplomer			Nucleocapsid			Envelope		
	Anti-TGEV	Anti-FIPV	Anti-CCV	Anti-TGEV	Anti-FIPV	Anti-CCV	Anti-TGEV	Anti-FIPV	Anti-CCV
TGEV	(0.91)	0.71	0.85	(0.99)	0.52	0.47	(0.97)	0.70	0.98
FIPV	1.00	(1.00)	0.95	1.00	(1.00)	1.00	1.00	(0.71)	0.84
CCV	0.94	0.87	(1.00)	0.89	0.42	(0.67)	0.86	1.00	(1.00)

[a] The highest absorbance value obtained in each block of nine reactions for one antigen served as the denominator in determining the ratios. Homologous reactions are in parentheses. Different sera have been used in this experiment as compared with Fig. 4.

one, and anti-CCV the weakest reaction. There were differences, however, in the degree to which cross-reactions with the different polypeptides occurred. Thus the P_{TGEV} and E_{TGEV} proteins were recognized by anti-TGEV and anti-FIPV sera to about the same extent, whereas canine anti-CCV serum showed only insignificant cross-reaction with the P_{TGEV} and N_{TGEV} antigens. Canine anti-CCV serum nevertheless strongly cross-reacted with the E_{TGEV} antigen (compare with the respective electroblot in Fig. 3).

Although cross-reactivity was clearly demonstrated, its degree cannot be assessed by this unilateral approach. Under the experimental conditions chosen, the antigenicity of a coronavirus protein in its host (the amount of antibody elicited by P, N, and E, respectively) is measured rather than the number of antigenic sites present on the homologous polypeptides. Therefore, we prepared the individual proteins of the three viruses and performed ELISA using gel eluates calculated to contain 5 μg of protein per ml. For a compact presentation (Table 2), the ratios of absorbance have been calculated between values obtained in the homologous and the heterologous reactions at a constant serum dilution (1:66); a value of 1.00 has been assigned to the highest ratio (usually the homologous reaction).

DISCUSSION

From the data presented, we conclude that the serological relationship between TGEV, FIPV, and CCV (17) is due to common antigenic determinants localized on the three major homologous coronavirus polypeptides. In vivo cross-protection studies (24) and tissue culture neutralization data (19, 22) had already indicated that a close relationship between TGEV and FIPV should exist at the level of the viral surface. The spike or peplomer glycoprotein (P), which carries determinants responsible for the induction of neutralizing antibody (3, 5), was recognized by heterologous serum in radioimmune precipitation-PAGE (Fig. 1 and 2), ELISA (Fig. 4 and Table 2), and (complement-independent) neutralization tests (Table 1). Unheated pig serum added to a mixture of TGEV and homologous antibody had no significant effect, whereas it clearly enhanced neutralization by feline anti-FIPV antibody. Fresh pig serum had been chosen as a complement source since it was reported not to affect pig cell-grown TGEV, whereas sera of other mammalian species reportedly contain complement-requiring (heterophile) antibody directed against porcine glycolipids (18). Indeed, part of the complement-requiring neutralizing activity could be absorbed out of the feline antibody preparations using porcine cell monolayers (18). In our experiments, pig complement consequently could have potentiated neutralization of (i) feline anti-porcine glycolipid antibody, (ii) feline anticoronavirus antibody at the level of the viral envelope (anti-E), or (iii) feline anticoronavirus antibody directed against the peplomeric surface projection (anti-P). To distinguish between the first and the two latter possibilities, TGEV grown in feline cells was assayed in neutralization mixtures supplemented with fresh porcine serum and feline serum, respectively. Again, neutralization enhancement was observed, notably also in the reaction where antibody, complement, and cells all were of feline origin. It is suggested that complement-requiring heterologous neutralization of TGEV by anti-FIPV antibody is caused by virolysis. From spatial considerations, the most likely structure involved in this process would be the membrane-embedded E protein. The virolysis phenomenon is well documented for other enveloped viruses, e.g., togaviruses (for a review see reference 8), and may well be of importance for virus neutralization in vivo.

The molecular weights of the N and E polypeptides of TGEV and CCV determined in this study agree with values published previously (4, 6); the P protein appeared distinctly smaller in our hands. The minor 21K protein of TGEV has not been described before. Depending upon the gel system, the envelope glycoprotein (E) may

be resolved into several fuzzy bands (Fig. 1 and 2) or appear homogenous (Fig. 3). Resolution of E_{CCV} into two separate polypeptides of 32K and 22K confirms recent results obtained by Garwes and Reynolds (6); since these authors were working with CCV grown in dog cells, the split low-molecular-weight glycoprotein does not appear to be a host cell-dependent phenotypic expression. The E glycoproteins constitute a major portion of the virion mass and elicit the most prominent antibody response (Fig. 4). This is in contrast to observations with human respiratory coronavirus 229E viruses, where most of the antibody made during experimental infection of volunteers was directed against the viral surface projection (13). The differences may be explained by our use of hyperimmune serum (TGEV) and the different pathogenesis of generalized (FIPV) and enteric (CCV) infections. Cross-reactivity at the E level has been demonstrated using radioimmune precipitation, electroblotting, and ELISA. The same techniques revealed common determinants at the level of the nucleocapsid protein (N). Since the reaction of porcine immunoglobulin with N_{TGEV} resulted in a non-dissociable complex (Fig. 2) unable to penetrate the polyacrylamide gel, a quantitative comparison was made using the ELISA technique.

As can be seen in Table 2, recognition of antigenic sites on the P and E proteins by heterologous antibody is comparable to that by homologous antibody (values approaching 1.00). Also, porcine anti-TGEV serum reacts with N_{FIPV} and N_{CCV} to about the same extent as with N_{TGEV}. In contrast, canine anti-CCV serum shows a weak reaction with N_{TGEV} as does feline anti-FIPV serum with the heterologous N proteins. Since all three sera react to a maximum degree with N_{FIPV}, the insufficient recognition by feline and canine antibody of heterologous determinants is conceivably due to differences in their antigenicity or to low avidity of the respective antibody.

In conclusion, the coronaviruses causing transmissible gastroenteritis in swine, peritonitis in felines, and gastroenteritis in dogs are very closely related. Cross-reaction using immunofluorescence and notably also virus neutralization tests (1, 17, 20, 22) have been established. The recent finding that virulent TGEV and FIPV produce fatal infections and indistinguishable morphological changes in the intestines of experimentally infected newborn piglets (23) supports this statement. In fact, the three viruses may be regarded as host range mutants rather than as different "species"; the E proteins may prove useful as identification aids in comparative analyses. It is anticipated that the hitherto monogeneric Coronaviridae family (21) will be subdivided; TGEV, FIPV, and CCV will be assigned to one taxonomic cluster, probably together with the human respiratory coronavirus strains 229E (17) and five additional human isolates (14). Hemagglutinating encephalitis (vomiting and wasting) virus of swine (17), neonatal calf diarrhea virus (7, 10), mouse hepatitis virus type 3, and the human isolates OC43, RO, HO, and G1 (14) may constitute a second cluster of mammalian coronaviruses. The taxonomic considerations may have evolutionary implications for the epidemiology of coronarvirus infections in animals and man.

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