Fatal Enteritis Associated with Coronavirus Infection in Cats

A. Kipar, J. Kremendahl, D. D. Addie*, W. Leukert, C. K. Grant† and M. Reinacher

*Institut für Veterinär-Pathologie, Universität Leipzig, Margarete-Blank-Strasse 4, 04103 Leipzig, Germany, *Department of Veterinary Pathology, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK, and †Custom Monoclonals International, 813 Harbor Boulevard 248, West Sacramento, CA 95691, USA*

Summary

This report describes five cases of naturally occurring feline coronavirus enteritis. The affected animals, aged 2 months to 7 years, had a clinical history of intestinal symptoms, including diarrhoea or vomiting, or both. They exhibited variable histological changes in the epithelium of the small intestine, ranging from degeneration of single cells and detachment of groups of cells from the villous tips to regenerative processes of the crypt epithelia. Post-mortem diagnosis was based on the immunohistochemical demonstration of coronavirus antigen within intestinal epithelial cells and on the electron microscopical demonstration of coronavirus particles in the faeces. In addition, one animal was immunohistochemically positive for antigens of feline leukaemia virus (FeLV) and exhibited intestinal changes consistent with FeLV-associated enteritis. Two cats were tested serologically for feline immunodeficiency antibodies, with negative results. The findings indicate that natural coronavirus infection is a potential cause of severe enteritis in juvenile and adult cats.   © 1998 W.B. Saunders Company Limited

Introduction

Feline enteric coronavirus (FECV), a potentially enteropathogenic ubiquitous feline coronavirus, and feline infectious peritonitis virus (FIPV) are regarded as two virulence variants of feline coronavirus (FCoV) (Pedersen and Floyd, 1985; Fiscus and Teramoto, 1987a, b; Herrewegh et al., 1995b). Unlike FIPV infection, FECV infection is not regarded as fatal (Evermann et al., 1991; Pedersen, 1995). FCoV is spread by the faecal-oronasal route (Hoshino and Scott, 1980a; Pedersen et al., 1981a; Hayashi et al., 1982; Stoddart et al., 1988; Herrewegh et al., 1995a; Pedersen, 1995; Vennema et al., 1995; Addie et al., 1996; Poland et al., 1996).

Information on feline coronavirus enteritis has been gained mainly by experimental studies on FECV and FIPV (Pedersen et al., 1981a; Hayashi et al., 1982; Pedersen et al., 1984; Stoddart et al., 1988). After oral administration, FECV induced severe enteritis in 6-week-old specific pathogen-free kittens, but older kittens remained healthy or developed only mild enteritis (Pedersen et al., 1981a). Kittens are protected by maternal antibodies until they are 5 to

6 weeks old; infections occur at 5 to 10 weeks of age (Pedersen, 1983; Addie and Jarrett, 1992).

Histologically, coronavirus enteritis is characterized by patchy changes in the gut, from the distal part of the duodenum to the terminal part of the ileum, in the form of villous atrophy of varying severity (Pedersen *et al.*, 1981a; Pedersen, 1987a). At the villous tips, fusion of adjacent epithelial cells as well as separation and sloughing of epithelial cells from the underlying basement membrane occur, the latter resulting in villous retraction. Focal fusion of adjacent villi is also observed. Regenerative processes are represented by deepening of crypts in some areas and covering of denuded villous tips by immature epithelium. Mild focal inflammatory reactions were observed by Pedersen *et al.* (1981a) and Pedersen (1987a). Expression of coronavirus antigen, demonstrated by immunofluorescence, was restricted to mature columnar epithelial cells and single monocytic cells in the mesenteric lymph node (Pedersen *et al.*, 1981a). In recovered cats, a few small foci of infected epithelial cells were often detected in small intestine and caecum (Pedersen, 1983, 1987a).

McKeirnan *et al.* (1981) isolated cytopathogenic coronavirus from the mesenteric lymph nodes and intestinal washings of a cat that had died with clinical signs of intermittent haemorrhagic diarrhoea and exhibited histological changes compatible with feline parvovirus infection. Dea *et al.* (1982) demonstrated coronavirus particles by electron microscopy in the diarrhoeic faeces of a cat which subsequently died. Hayashi *et al.* (1982) demonstrated coronavirus antigen in four cats suffering from enteritis, with or without peritonitis.

This report describes five cases of natural coronavirus enteritis mimicking the variety of changes observed in kittens experimentally infected with FECV (Pedersen *et al.*, 1981a). In one case, feline leukaemia virus (FeLV) co-infection and intestinal changes compatible with FeLV-associated enteritis (Reinacher, 1987) were also demonstrated.

Materials and Methods

Animals and Tissue Processing

Naturally infected cats (nos 1–5), presented to veterinary surgeons for diagnosis and treatment in Germany (nos 1, 2 and 4) or the UK (nos 3 and 5), were subjected to post-mortem examination within a few hours of death. From cat 3, only part of the small intestine was available for histological examination. Further details of the animals are given later. Tissues were fixed in 10% non-buffered formalin for 12 to 18 h and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE), Gram stain (modified by Weigert) or Warthin-Starry stain; others were used for immunohistochemical examination.

Electron Microscopy

Faecal samples of cats 1 and 2 were examined for viral particles after negative staining (Arens and Krauss, 1980; Vieler and Herbst, 1995). Briefly, samples were diluted with an equal volume of 0·01 M phosphate buffer containing NaCl 0·83% (pH 7·2) and incubated for 1 h at room temperature, followed by centrifugation at 2000 *g* for 15 min. One drop of supernate was dried on a grid and negatively stained for 5 min with 3% phosphotungstic acid (pH 5.6).

Immunohistochemical Examination

Tests were made on the jejunum (cats 1–5), and mesenteric lymph node, spleen and bone marrow (cats 1, 2, and 3). From cat 5, gastric, duodenal, ileal, colonic and rectal specimens were also examined. The indirect peroxidase and the peroxidase anti-peroxidase (PAP) tests were made as described previously (Sternberger *et al.*, 1971; Kovacevic *et al.*, 1997). Briefly, sections were dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase was blocked by incubation with hydrogen peroxide 0·3% in methanol at room temperature for 30 min. Sections were washed with Tris-buffered saline (TBS; 0·1M Tris-HCl, NaCl 0·8%, pH 7·6) and treated with "target unmasking fluid" (TUF; Dianova GmbH, Hamburg, Germany) for 10 min at 96°C. Rat serum 10% (or for FeLV antigen demonstration, swine serum 50%) in TBS was applied for 10 min at room temperature before treatment with the primary antibodies. Specimens were then incubated for 12 to 16 h at 4°C with the following primary antisera: mouse anti-coronavirus (FCV3-70; Custom Monoclonals International, West Sacramento, USA) 1 in 100 in TBS; mouse anti-canine parvovirus (Dr A. Aubert, Virbac Laboratories, Carros Cedex, France) 1 in 1000 in TBS; mouse anti-FeLV gp70 (C11D82i; Custom Monoclonals International) 1 in 200 in TBS containing swine serum 20%; mouse anti-FeLV p27 (PF12J-10A; Custom Monoclonals International) 1 in 100 in TBS containing swine serum 20%; and mouse anti-FeLV p15E (PF6J-2A; Custom Monoclonals International) 1 in 100 in TBS containing swine serum 20%. To demonstrate antigens other than those of FeLV, rat anti-mouse IgG (Dianova; 1 in 100 in TBS) and mouse PAP complex (Dianova; 1 in 500 in TBS) were applied. FeLV antigens were detected by incubation with peroxidase-labelled rabbit anti-mouse IgG (Dako Diagnostika GmbH, Hamburg, Germany; 1 in 100 in TBS). Between each 30-min incubation step, slides were washed with TBS. Sections were incubated for 10 min with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Serva, Heidelberg, Germany) 0·05% in 0·1M buffered imidazole/HCl (pH 7·1) and counterstained with Papanicolaou's haematoxylin (E. Merck, Darmstadt, Germany; 1 in 20 in distilled water).

Formalin-fixed and paraffin wax-embedded intestinal, lymphoid and bone marrow specimens from 10 cats with feline panleukopenia, 10 FeLV-positive cats with FeLV-associated enteritis and 10 FeLV-positive cats without intestinal alterations served as positive controls for parvovirus and FeLV antigen (Reinacher, 1987; Kovacevic *et al.*, 1997). In addition, preparations from granulomatous-necrotizing lesions of 10 cats with feline infectious peritonitis (in different organs and tissues, including peritoneum and kidneys) served as positive controls for coronavirus antigen (Tammer *et al.*, 1995); from these cats, intestinal, lymphoid and bone marrow specimens were also examined. To control for non-specific reactions, tissue specimens from 10 FeLV-negative cats exhibiting various abnormalities other than coronavirus enteritis or FIP, and showing no clinical signs of enteritis or histopathological changes in the small intestine, were examined. Furthermore, a non-reacting mouse monoclonal antibody directed against rat pyruvate kinase isoenzyme type L was used on sections from each block to control for non-specific binding of secondary and tertiary antibodies (Domingo *et al.*, 1992).

Test for Feline Coronavirus Antibodies

An immunofluorescent antibody technique was used, as described by Addie and Jarrett (1992).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for Detection of Coronavirus RNA

The RT-PCR method used was as described previously (Herrewegh *et al.*, 1995a; Addie *et al.*, 1996).

Table 1
Main findings in five cats

Cat no.	Clinical signs	Gross pathology	Histopathology	Immunohistochemistry
1	Diarrhoea, vomiting	Gastroenteritis	Intestine: shortened villi, sloughing and degeneration of epithelial cells, epithelial regeneration, abundant bacteria in crypts. Spleen: bacteria in red pulp. Bone marrow: hypercellular, agglomerations of lymphocytes and bacteria.	Intestine: epithelial cells coronavirus-positive
2	Diarrhoea, cachexia, respiratory signs, tremor, flea infestation, anaemia	Cachexia, lymphadenopathy, flea infestation, anaemia	Intestine: shortened villi, sloughing and degeneration of epithelial cells. Bone marrow: hypercellular.	Intestine: epithelial cells coronavirus-positive; FeLV-positive
3	Polyphagia, frequent defecation, respiratory signs, eczema	Ascites, lymphadenopathy, cachexia, anaemia	Intestine: crypt epithelium hyperplasia, villous epithelium degeneration. Lymph nodes: sinus histiocytosis, follicular hyperplasia. Bone marrow: hypercellular.	Intestine: epithelial cells coronavirus-positive
4	Anorexia, vomiting, leucocytosis	No obvious changes	Intestine: sloughing and degeneration of epithelial cells, fusion of villi.	Intestine: epithelial cells coronavirus-positive
5	Diarrhoea, vomiting, weight loss	No obvious changes	Intestine: shortened villi, fusion of villi, sloughing and degeneration of epithelial cells.	Intestine: epithelial cells coronavirus-positive

Results

The main findings (clinical, pathological, histopathological and immuno-histochemical) are summarized in Table 1.

Cat No. 1

This 2-month-old male domestic short-haired cat from a single-cat household was painlessly killed because the clinical signs of diarrhoea and vomiting had led to a presumptive diagnosis of feline panleukopenia. Information about vaccinations was not available. At gross examination, the animal showed moderate emaciation, acute diffuse catarrhal gastroenteritis and red-stained bone marrow.

Histopathologically, shortened small intestinal villi, with tips exhibiting necrosis and sloughing or absence of epithelial cells, were seen. The crypts were almost denuded of epithelial cells, or were dilated and contained flattened
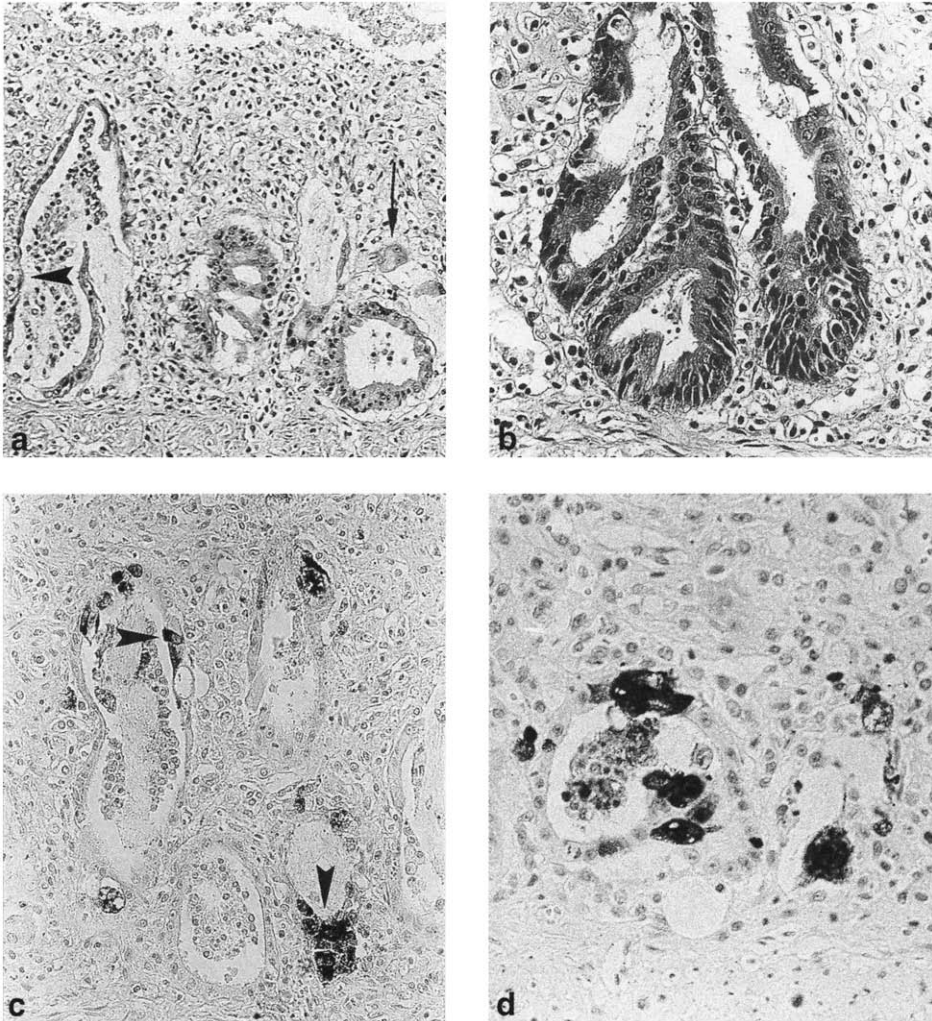
Fig. 1. Cat 1. Jejunum. (a) Dilation of crypts, flattening of crypt epithelial cells (arrowhead), and intraluminal detritus. Note the presence of enteroblasts (arrow), and marked mononuclear infiltration of the mucosa. HE. × 130. (b) Hyperplasia of crypt epithelial cells. HE. × 150. (c) Coronavirus antigen-positive crypt epithelial cells (arrowheads). PAP method, Papanicolaou's counterstain. × 180. (d) Coronavirus antigen-positive enteroblasts in intestinal crypts. PAP method, Papanicolaou's counterstain. × 280.

epithelial cells, single necrotic cells, and detritus [Fig. 1(a)]. Some crypts, however, showed moderate to distinct epithelial hyperplasia and single enteroblasts [Fig. 1(a),(b)]. The mucosa and submucosa were markedly infiltrated by mononuclear cells [Fig. 1(a)], and a few infiltrating cells were scattered in the muscularis. In the spleen, agglomerations of predominantly Gram-negative rod-shaped bacteria were observed, disseminated in the red pulp. Lymphoid follicles were unaltered. The bone marrow was markedly hypercellular with numerous blast cells; in addition, focal accumulations of lymphocytes and clumps of predominantly Gram-negative rod-shaped bacteria were found.
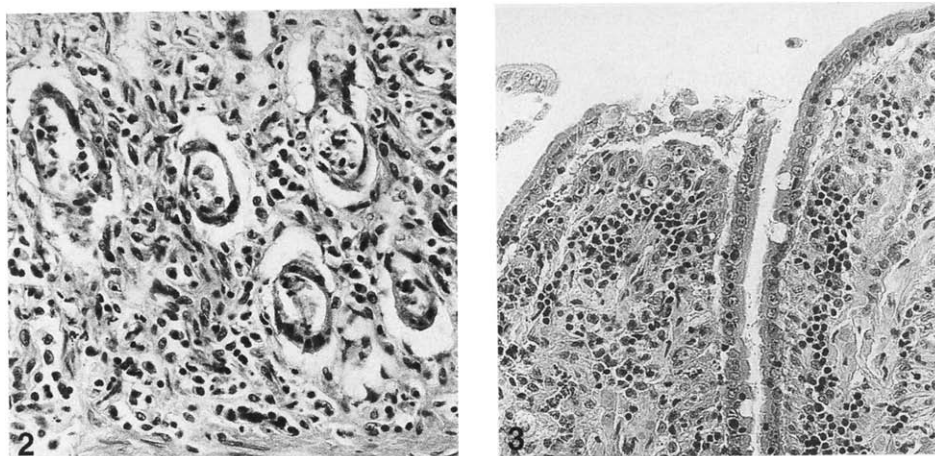
Fig. 2.   Cat 2. Jejunum. Numerous degenerate crypt epithelial cells, detritus within crypt lumina, and
          moderate mononuclear infiltration of the mucosa. HE. × 240.
Fig. 3.   Cat 3. Jejunum. Focal degeneration with detachment of villous tip epithelial cells. Moderate
          mononuclear infiltration of the mucosa. HE. × 240.

Immunohistochemically, very few epithelial cells at the villous tips or detached from the villi were labelled for coronavirus antigen. In some crypts, variable numbers of epithelial cells, connected with or detached from the crypt wall, showed a positive reaction [Fig. 1(c)]. Detritus within dilated crypts was occasionally positively labelled, as also were some hypertrophic crypt epithelial cells and enteroblasts [Fig. 1(d)]. Coronavirus antigen was not detected in mesenteric lymph node, spleen or bone marrow. All tissues examined were negative for antigens of parvovirus and FeLV.

Electron microscopically, coronavirus particles were detected in the faeces. They were seen as pleomorphic spherical virions, approximately 100 nm in diameter, exhibiting large petal-shaped spikes.

Cat No. 2

This 8-month-old male, non-vaccinated short-haired cat had shown recurrent diarrhoea, cachexia and upper respiratory tract disease for 6 months. Clinically, anaemia, hypothermia, tremor, vomiting, haemorrhagic diarrhoea and flea infestation were noted, and the cat was painlessly killed. At necropsy, the animal showed severe anaemia and emaciation, and moderate enlargement of all lymph nodes. The gut appeared normal.

Histopathologically, the small intestine showed shortened villi, with degeneration and sloughing of variable numbers of epithelial cells at the tips. The crypts also exhibited degenerate epithelial cells and were sometimes dilated by detritus (Fig. 2). The mucosa was moderately infiltrated by mononuclear cells. The mesenteric lymph nodes and spleen were moderately congested and contained small collagen-fibre scars in the centre of lymphoid follicles. The latter showed a reduced number of cells. In the spleen, moderate

extramedullary haematopoiesis was found, including megakaryocytes in the red pulp. The bone marrow contained numerous blast cells.

Immunohistochemically, several coronavirus antigen-positive epithelial cells were found detached from the villi and at the villous tips. Additionally, moderate labelling of crypt epithelial cells and infiltrating lymphocytes for FeLV gp70 and p15E were observed. Labelling for p27 of FeLV was only faint. Within lymphoid follicles of mesenteric lymph node and spleen, varying numbers of cells positive for all three FeLV antigens were found. In bone marrow, moderate labelling for gp70 was observed within megakaryocytes. All tissues examined were negative for parvovirus antigen.

Electron microscopy revealed coronavirus particles in the faeces.

Cat No. 3

Aged 18-months, this male castrated short-haired cat had been housed in an animal shelter for at least 6 months, where it had been vaccinated against upper respiratory disease viruses and parvovirus, and regularly treated against ecto- and endo-parasites. The animal had a clinical history of recurrent respiratory symptoms and eczema-like skin lesions. For 4 months the cat had shown polyphagia and increased defecation. Six weeks before its death, it was found to be serologically negative for FeLV p27 and feline immunodeficiency virus (FIV) antibodies. On post-mortem examination, cachexia, moderate anaemia and ascitic fluid (25 ml) were found. Lymph nodes were generally enlarged. Moderate numbers of *Escherichia coli* and enterococci were isolated from the mesenteric lymph nodes, liver and lungs. Attempts to culture cytopathogenic viruses on Crandell feline kidney cells (ATCC-No. CCL 94) from homogenized lung, liver, kidney and spleen tissue suspensions proved unsuccessful.

The small intestinal crypt epithelia were moderately hyperplastic. Villous epithelia exhibited focal degeneration at both margins and tips (Fig. 3). Additionally, moderate follicular hyperplasia of Peyer's patches, mononuclear plasma cell-dominated infiltration of the mucosa, and moderate mucosal oedema were observed. Mesenteric lymph nodes showed massive histiocytosis of sinusoids, follicular hyperplasia with numerous sinusoidal plasma cells, and degeneration of some cells in the follicular centres. The spleen exhibited moderate congestion, moderate lymphocytic depletion, and degeneration of intrafollicular cells. The bone marrow was markedly hypercellular. The lungs showed congestion, focal acute alveolar emphysema, moderate hypertrophy of the bronchial muscularis, and hyperplasia of bronchial epithelia. The myocardium showed slight mononuclear interstitial infiltrates. The liver was congested, with mononuclear plasma cell-dominated interstitial hepatitis, fat storage within Ito cells, bile pigment within hepatocytes, moderate congestion, and degeneration of some hepatocytes. Chronic mononuclear interstitial nephritis with focal proliferation of fibrous tissue, and moderate congestion and oedema of the neuropil, were also observed.

Immunohistochemical examination for coronavirus antigen showed several positive epithelial cells detached from the jejunal villi, and small numbers at

the villous tips; Lymphoid tissues and bone marrow were negative. Parvovirus and FeLV antigens were not detected in any tissue.

Cat No. 4

This animal was a spayed Persian cat aged 12 months that had suffered from chronic anorexia and occasional vomiting for 3 months and died after a short period of pharyngitis. Haematological examination had revealed a high white blood cell count. The animal was serologically negative for FeLV p27 antigen and FIV antibodies. Macroscopically, no obvious abnormalities were observed.

   Histological changes included foci in which villous epithelial cells were absent, and fusion of villi in the small intestine. Villous tip epithelia showed degeneration of single cells and occasionally sloughing of cell groups [Fig. 4(a)]. Small numbers of mononuclear cells were scattered in the moderately oedematous mucosa.

   Coronavirus antigen was demonstrated within single epithelial cells at the small intestinal villous tips [Fig. 4(b)]. Parvovirus and FeLV antigens were not detected.

Cat No. 5

This 7-year-old male Havanna cat had shown chronic weight loss and recurrent diarrhoea and vomiting for 13 months. The coronavirus antibody titre was 320 during the first period of diarrhoea and 1280 at the time of euthanasia. At the same times the RT-PCR demonstrated that coronavirus was present in faeces but absent from saliva. At necropsy, no macroscopical abnormalities were seen.

   Histological examination revealed focal fusion of villi, mild villous atrophy and degeneration and sloughing of epithelial cells at the tips of the villi, mainly in the duodenum and ileum. The small intestine showed moderate oedema of the intestinal wall and mononuclear infiltration of the mucosa. The colon and rectum showed moderate mononuclear infiltration of the mucosa and submucosa. The mesenteric lymph nodes were moderately oedematous.

   Coronavirus antigen was present in some epithelial cells at the villous tips in the jejunum and ileum, and in some detached cells. Parvovirus and FeLV antigens were not detected.

Control Cats

In cats with FIP, labelling of coronavirus antigen was restricted to macrophages within granulomatous necrotizing lesions. Intestinal epithelia showed no changes resembling those in cats 1 to 5 and were negative for coronavirus, parvovirus and FeLV antigens. In cases of FeLV-associated enteritis, histological changes in small intestinal crypt epithelia were comparable with those observed in cat 2. Villous tip epithelia were unaltered. The labelling pattern for FeLV antigens in intestinal epithelial cells was similar to that in cat 2, i.e., labelling was intense for gp70 and p15E but weak for p27. In FeLV-positive
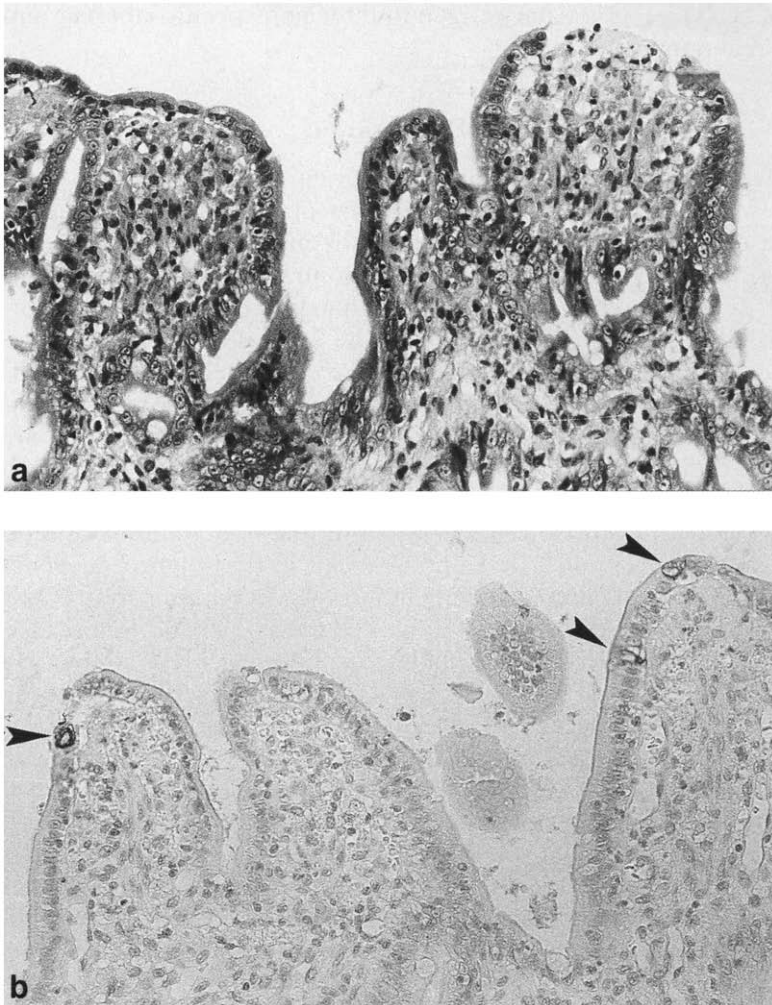
Fig. 4. Cat 4. Jejunum. (a) Fusion of villi and foci in which villous tip epithelial cells are absent. Moderate oedema and mononuclear infiltration of the mucosa. HE. × 240. (b) Isolated coronavirus antigen-positive epithelial cells at the villous tips (arrowheads). PAP method, Papanicolaou's counterstain. × 270.

cats without enteritis, the small intestinal epithelia were unaltered. Intestinal epithelial cells were labelled strongly for FeLV p27 but only weakly for gp70 and p15E. In both groups of cases, lymphoid tissues and bone marrow showed labelling patterns for FeLV antigens which were comparable with that described for cat No. 2. Neither coronavirus antigen nor parvovirus antigen was detected in any animal.

In cats with panleukopenia, the epithelial cells at the villous tips appeared normal. Intact crypt epithelial cells contained parvovirus antigen, but villous epithelial cells were negative. Neither coronavirus antigen nor FeLV antigen was detected.

Negative controls for each antigen and for non-specific labelling showed no positive reactions.

Discussion

This report describes five cases of naturally occurring feline coronavirus enteritis in cats from 2 months to 7 years of age. Like previous reports of single cases of natural coronavirus enteritis in cats (McKeirnan *et al.*, 1981; Dea *et al.*, 1982; Hayashi *et al.*, 1982), it contrasts with experimental studies, in which severe enteritis and intestinal changes could be induced only in 6-week-old kittens (Pedersen *et al.*, 1981a, b). Jejunal lesions (degeneration and sloughing of villous-tip epithelial cells) were consistently observed. Two cats exhibited signs of epithelial regeneration (hyperplasia of crypt epithelia). In one cat, enteroblasts were present; this animal was the only one with a presumptive diagnosis of feline panleukopenia. The histological changes observed in our cases resembled those previously reported in a 6-week-old kitten which developed severe enteritis 3 days after the oral administration of a cell-free ileal extract from an FECV-infected cat; in that report, deepened crypts and immature epithelium at the villous tips were interpreted as signs of regeneration (Pedersen *et al.*, 1981b; Pedersen, 1987a). Identical changes, however, can be induced even in older cats by some FIPV strains (Hayashi *et al.*, 1982; Stoddart *et al.*, 1988). Hayashi *et al.* (1982) described slight shortening of villi, oedema in the lamina propria, and infiltration of neutrophils and mononuclear cells, within 24 h of intragastric infection of kittens with a liver emulsion from an FIPV-infected cat (Yayoi strain). From day 3 post-infection (pi) onwards, changes consistent with those described for coronavirus enteritis (Pedersen et al., 1981b) were observed, and regenerative processes first occurred on day 5. Animals that died on day 5 or 6 pi exhibited severe erosion, haemorrhage and inflammation of the lamina propria. From day 0·5 to day 12 pi, FIPV antigen-positive enterocytes were detected by immunofluorescence. All animals had diarrhoea. Stoddart *et al.* (1988) orally infected five 9-month-old cats with the "Wellcome" strain, cultured on a feline embryonic lung cell line. Animals were killed at days 1, 2, 3, 7 or 14 pi without having developed diarrhoea. Intestinal changes (blunting and fusion of villi in the distal small intestine) were seen only at day 14 pi, in an animal in which FIPV antigen was restricted to the caecum and colon; cats killed from days 1–7 pi showed viral antigen in addition in the small intestine. In contrast, we found coronavirus antigen-positive cells in the jejunum of all animals, but the colonic epithelium was negative in the one cat in which it was examined.

Diagnosis of coronavirus enteritis was confirmed by immunohistochemical demonstration of coronavirus antigen in small intestinal epithelial cells and supported by the electron microscopical demonstration of coronavirus in the faeces of two cats and by RT-PCR in another. Parvovirus infection was excluded immunohistochemically in all cases. Cats without enteritis showed no positive reaction for coronavirus antigen, but those with enteritis showed coronavirus antigen-positive cells at the villous tips or detached from the villi. Furthermore, the cat with distinct regenerative changes exhibited a positive

reaction in flattened and hypertrophic crypt epithelial cells as well as in enteroblasts, indicating that immature intestinal epithelial cells can be infected by coronaviruses. These results differed from those of a previous study in cats experimentally infected with FECV, in which coronavirus antigen was demonstrated only in mature columnar epithelia (Pedersen *et al.*, 1981b, 1984; Pedersen, 1983). However, Hoshino and Scott (1980a, b) demonstrated in specific pathogen-free kittens infected with FIPV that coronavirus replicates in the cytoplasm of villous epithelial cells. Three cats examined in the present study had shown diarrhoea lasting for 3 to 6 months. Bearing in mind the relatively short period in which coronavirus antigen was detectable after experimental FIPV infection (Hayashi *et al.*, 1982; Stoddart *et al.*, 1988), the question arises as to whether the diarrhoea in the present study was due to a persistent coronavirus infection of intestinal epithelia.

In cat 2, coronavirus and FeLV co-infection was demonstrated, the crypt epithelia showing mild changes consistent with FeLV-associated enteritis (Reinacher, 1987). FeLV infection was confirmed immunohistochemically, increased expression of envelope proteins gp70 and p15E by crypt epithelial cells being a consistent finding in FeLV-associated enteritis (Kremendahl *et al.*, 1996). The findings (histopathological and immunohistochemical) for FeLV and coronavirus antigens were, however, clearly discernible from each other, indicating that they developed independently. In cat 2, FeLV may have activated an inapparent, in this case intestinal, coronavirus infection, as suggested by Pedersen (1987b). Moreover, degeneration of FeLV-infected crypt epithelial cells may have contributed to the fatal outcome of intestinal coronavirus infection (Reinacher, 1987). Evermann *et al.* (1991) speculated as to the effect of concurrent virus infections on the development of clinical signs in coronavirus enteritis.

In two of the present cases (nos 1 and 3), *E. coli* was found in tissues by microbiological examination, or its presence was inferred from the histological demonstration of Gram-negative rod-shaped bacteria in both spleen and bone marrow. These results indicated that the animals had developed a terminal bacteriaemia.

Previous reports on spontanous feline coronavirus enteritis are few in number. McKeirnan *et al.* (1981) described an 18-month-old female domestic short-haired cat showing a striking loss of small intestinal villous epithelial cells, with necrotic cells, haemorrhagic foci, dilated crypts and shortened villi covered with bacteria. The bone marrow was depleted of haematopoietic cells and the lymphoid tissues exhibited lymphoid depletion. Cytopathogenic coronavirus was isolated from mesenteric lymph nodes and intestinal washings, but the authors suspected a concurrent parvovirus infection from the type of intestinal changes. By electron microscopy, Dea *et al.* (1982) demonstrated coronavirus particles in the faeces of an 8-month-old cat that died after having suffered from diarrhoea for 2 days; the animal was not subjected to post-mortem examination and therefore the presumptive diagnosis of coronavirus enteritis could not be confirmed. Hayashi *et al.* (1982) described three cats with ascites and fibrinous serositis suggestive of FIP, and mild changes consistent with coronavirus enteritis, and a fourth, aged 2·5 years, with

coronavirus enteritis-type changes alone; all animals had FCoV antibodies, and FCoV antigen was demonstrated by immunofluorescence in enterocytes.

As already stated, FIPV and FECV are regarded as virulence variants of FCoV. Due to antigenic and cultural characteristics, however, FCoV strains are further divided into serotypes I and II, each comprising both FECV and FIPV strains (Pedersen and Floyd, 1985; Fiscus and Teramoto, 1987a, b; Hohdatsu *et al.*, 1991, 1993; Horzinek *et al.*, 1995; Vennema *et al.*, 1995). The precise genetic basis for the differences in virulence between FIPV and FECV is not yet known (Vennema *et al.*, 1995; Poland *et al.*, 1996). However, the demonstration of closely related FECV and FIPV strains in the same geographical region or cattery, together with the greater genetic differences that appear to result from geographical and temporal separation, suggests that FIPV arises from FECV and that the two viruses do not arise from separate lineages (Vennema *et al.*, 1995). FIPV mutants have been isolated from FIV-positive cats with FIP that had been experimentally infected with FECV (Poland *et al.*, 1996), and, moreover, coronaviruses have a high degree of inherent mutability (Horzinek *et al.*, 1995). It would seem possible that FIPV represents the outcome of continuous mutation from FECV (Pedersen *et al.*, 1981b; Pedersen, 1995).

This study shows that naturally occurring virulent feline coronavirus infection not only induces FIP but is a potential cause of severe enteritis in juvenile and adult cats. The widespread distribution of coronavirus infection in cat populations may lead to the sporadic occurrence of enteritis (Addie and Jarrett, 1992). As the immunohistochemical results showed, coronavirus antigen is almost exclusively confined to epithelial cells at, or detached from, the villous tips. This limits immunohistochemical diagnosis of coronavirus enteritis to carcasses that are fresh enough to be free of abundant post-mortem sloughing of intestinal epithelial cells at the villous tips.

References

Addie, D. D. and Jarrett, O. (1992). A study of naturally occurring feline coronavirus infections in kittens. *Veterinary Record*, **130**, 133–137.

Addie, D. D., Toth, S., Herrewegh, A. and Jarrett, O. (1996). Feline coronavirus in the intestinal contents of cats with feline infectious peritonitis. *Veterinary Record*, **139**, 522–523.

Arens, M. and Krauss, H. (1980). Detection of parvovirus in dogs with acute gastroenteritis. *Berliner Münchner Tierärztliche Wochenschrift*, **93**, 156–157.

Dea, S., Roy, R. S. and Elazhary, M. A. S. Y. (1982). Coronavirus-like particles in the feces of a cat with diarrhea. *Canadian Veterinary Journal*, **23**, 153–155.

Domingo, M., Einig, C., Eigenbrodt, E. and Reinacher, M. (1992). Immunohistological demonstration of pyruvate kinase isoenzyme type L in rat with monoclonal antibodies. *Journal of Histochemistry and Cytochemistry*, **40**, 665–673.

Evermann, J. F., McKeirnan, A. J. and Ott, R. L. (1991). Perspectives on the epizootiology of feline enteric coronavirus and the pathogenesis of feline infectious peritonitis. *Veterinary Microbiology*, **28**, 243–255.

Fiscus, S. A. and Teramoto, Y. A. (1987a). Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. *Journal of Virology*, **61**, 2607–2613.

Fiscus, S. A. and Teramoto, Y. A. (1987b). Functional differences in the peplomer glycoproteins of feline coronavirus isolates. *Journal of Virology*, **61**, 2655–2657.

Hayashi, T., Watabe, Y., Nakayama, H. and Fujiwara, K. (1982). Enteritis due to feline infectious peritonitis virus. *Japanese Journal of Veterinary Science*, **44**, 97–106.

Herrewegh, A. A. P. M., de Groot, R. J., Cepica, A., Egberink, H. F., Horzinek, M. C. and Rottier, P. J. M. (1995a). Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. *Journal of Clinical Microbiology*, **33**, 684–689.

Herrewegh, A. A. P. M., Vennema, H., Horzinek, M. C., Rottier, P. J. M. and de Groot, R. J. (1995b). The molecular genetics of feline coronaviruses: comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virology*, **212**, 622–631.

Hohdatsu, T., Okada, S. and Koyama, H. (1991). Characterization of monoclonal antibodies against feline infectious peritonitis virus type II and antigenic relationship between feline, porcine, and canine coronaviruses. *Archives of Virology*, **117**, 85–95.

Hohdatsu, T., Yamada, H., Ishizuka, Y. and Koyama, H. (1993). Enhancement and neutralization of feline infectious peritonitis virus infection in feline macrophages by neutralizing monoclonal antibodies recognizing different epitopes. *Microbiological Immunology*, **37**, 499–504.

Horzinek, M. C., Herrewegh, A. and de Groot, R. J. (1995). Perspectives on feline coronavirus evolution. *Feline Practice*, **23**, 34–39.

Hoshino, Y. and Scott, F. W. (1980a). Coronavirus-like particles in the faeces of normal cats. *Archives of Virology*, **63**, 147–152.

Hoshino, Y. and Scott, F. W. (1980b). Immunofluorescent and electron microscopic studies of feline small intestinal organ cultures infected with feline infectious peritonitis virus. *American Journal of Veterinary Research*, **41**, 672–681.

Kovacevic, S., Kipar, A., Kremendahl, J., Teebken-Schuler, D., Grant, C. K. and Reinacher, M. (1997). Immunohistochemical diagnosis of feline leukemia virus infection in formalin-fixed tissue. *European Journal of Veterinary Pathology*, **3**, 13–19.

Kremendahl, J., Kipar, A. and Reinacher, M. (1996). Examinations on the differential expression of viral proteins gp70, p27, and p15(E) in spontaneous FeLV-associated enteritis. *European Journal of Veterinary Pathology*, **2**, Suppl., 21.

McKeirnan, A. J., Evermann, J. F., Hargis, A., Miller, L. M. and Ott, R. (1981). Isolation of feline coronaviruses from cats with disease manifestations. *Feline Practice*, **11**, 16–20.

Pedersen, N. C. (1983). Feline infectious peritonitis and feline enteric coronavirus infections. Part 1: Feline enteric coronaviruses. *Feline Practice*, **13**, 13–18.

Pedersen, N. C. (1987a). Coronavirus diseases (coronavirus enteritis, feline infectious peritonitis). In: *Diseases of the Cat*, J. Holzworth, Ed., W. B. Saunders, Philadelphia, pp. 193–214.

Pedersen, N. C. (1987b). Virologic and immunologic aspects of feline infectious peritonitis virus infection. *Advances in Experimental Medicine and Biology*, **218**, 529–550.

Pedersen, N. C. (1995). An overview of feline enteric coronavirus and infectious peritonitis virus infections. *Feline Practice*, **23**, 7–20.

Pedersen, N. C. and Floyd, K. (1985). Experimental studies with three new strains of

feline infectious peritonitis virus: FIPV-UCD2, FIPV-UCD3, and FIPV-UCD4. *Compendium of Continuing Education*, **7**, 1001–1011.

Pedersen, N. C., Boyle, J. F. and Floyd, K. (1981a). Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. *American Journal of Veterinary Research*, **42**, 363–367.

Pedersen, N. C., Boyle, J. F., Floyd, K., Fudge, A. and Barker, J. (1981b). An enteric coronavirus infection of cats and its relationship to feline infectious peritonitis. *American Journal of Veterinary Research*, **42**, 368–377.

Pedersen, N. C., Evermann, J. F., McKeirnan, A. J. and Ott, R. L. (1984). Pathogenicity studies of feline coronavirus isolates 79–1146 and 79–1683. *American Journal of Veterinary Research*, **45**, 2580–2585.

Poland, A. M., Vennema, H., Foley, J. E. and Pedersen, N. C. (1996). Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *Journal of Clinical Microbiology*, **34**, 3180–3184.

Reinacher, M. (1987). Feline leukemia virus-associated enteritis – a condition with features of feline panleukopenia. *Veterinary Pathology*, **24**, 1–4.

Sternberger, L., Hardy, P., Cuculis, J. and Meyer, H. (1971). The unlabelled antibody-enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody-complex (horseradish peroxidase-antihorseradish-peroxidase) and its use in identification of spirochetes. *Journal of Histochemistry and Cytochemistry*, **18**, 315–333.

Stoddart, M. E., Gaskell, R. M., Harbour, D. A. and Pearson, G. R. (1988). The sites of early viral replication in feline infectious peritonitis. *Veterinary Microbiology*, **18**, 259–271.

Tammer, R., Evensen, O., Lutz, H. and Reinacher, M. (1995). Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Veterinary Immunology and Immunopathology*, **49**, 177–182.

Vennema, H., Poland, A., Floyd Hawkins, K. and Pedersen, N. C. (1995). A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. *Feline Practice*, **23**, 40–44.

Vieler, E. and Herbst, W. (1995). Detection of viruses in faeces of diarrhoeic dogs by electron microscopy. *Tierärztliche Praxis*, **23**, 66–69.