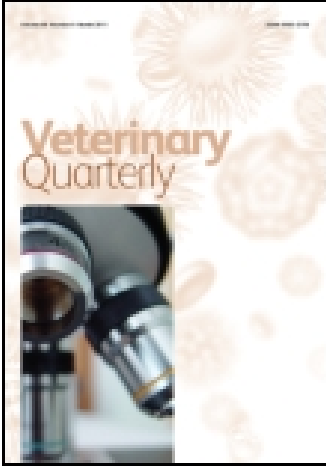


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Coronavirus infection in a litter of pups

J. Vandenberghe, R. Ducatelle, P. Debouck and J. Hoorens¹

SUMMARY

An outbreak of a coronavirus infection in a litter of 10-week-old poodle pups from a commercial kennel is described. Coronavirus-like particles were demonstrated by electron microscopy in the intestinal contents and in the colonic mucosa. Treponemas and campylobacters were excreted in great numbers in the faeces. The gastro-intestinal disturbances were complicated by hepatic and renal degeneration and severe bronchopneumonia.

INTRODUCTION

A coronavirus has been associated with gastro-intestinal disturbances in dogs (1, 3). The clinical signs of coronavirus infection are: sudden illness, lethargy, decrease of appetite, diarrhoea, and vomiting (2). The faeces have a fetid odor and may contain variable amounts of blood. Most animals recover spontaneously within 10 days. Symptomatic treatment shortens the recovery period. Loose stools can persist for 3 to 4 weeks. Leucopenia is not a common feature. Most infections pass subclinically (2).

Seventy per cent of dogs of all ages have seroneutralizing antibodies to T.G.E.-virus, a coronavirus in pigs (17). A possible relation between canine and porcine coronavirus infections has been mentioned by Albrecht and Lüpcke (1) and by Mc Clurkin *et al.* (13).

MATERIAL AND METHODS

Three poodle pups, 10 weeks old, from the same litter were brought to the veterinary faculty for examination. Their history was: sudden illness, lack of appetite, and death after a short period of listlessness and groaning. Profuse watery diarrhoea had been noticed for one day.

The dogs came from a commercial kennel of about fifty salable young dogs of all breeds. About 50 per cent of all pups in the kennel developed rhinitis and pneumonia and about 20 per cent developed diarrhoea, though mortality was low. All kennel dogs had been vaccinated with measles vaccine at the age of 7 weeks.

A visit to the kennel revealed satisfactory hygienic and alimentary conditions.

Three further poodle pups of the same litter, suffering from loose stools for one day, were brought to the laboratory for examination.

After clinical observations and routine blood and serum analyses the dogs were euthanized the same day.

Pathological examination

A detailed post-mortal examination was carried out. Tissue samples were fixed in 10 per cent phosphate-buffered formol saline for histological examination. Tissue of the middle jejunum and cranial part of the colon was fixed in 2.5 per cent glutaraldehyde and 2 per cent paraformaldehyde in 0.1 M cacodylate buffer for electron-microscopical examinations with a Zeiss EM 9.

Virological examination

A 20 per cent suspension of the colonic contents was prepared in phosphate-buffered saline. The supernatant of this suspension, obtained after low-speed centrifugation, was processed for electron microscopical examination as fully described elsewhere (18).

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Bacteriological examination

The faeces were examined for *Salmonella*, *Clostridium perfringens*, *Campylobacter jejuni*, and *Treponema*.

Routine technics were used for the isolation of *Salmonella*, namely enrichment on tetrathionate fluid medium, isolation on Gassner and brilliant green media, and the determination on Kligler medium.

Sheep blood agar plates were inoculated with the colonic contents for the isolation of *Clostridium perfringens* and incubated at 37° C in a BBL anaerobic system with catalyst for 24 hours.

For the isolation of *Campylobacter jejuni* a microaerophilic atmosphere was used, as described by Lawson and Rowland (12). Samples of the stomach, the middle jejunum, the colon, and the faeces were inoculated on Columbia sheep blood agar plates with 50 µg/ml actidione, 24 I.U./ml bacitracin, 15 µg/ml cephalotin, 10 U/ml colistin and 5 µg/ml novobiocin (11). Fecal samples of five other dogs with loose stools from the same kennel were examined for *Campylobacter jejuni*.

Rectal samples were inoculated, for the isolation of *Treponema*, on Columbia sheep blood agar plates with 180 I.U./ml polymyxin B sulphate (6) or 400 µg/ml spectinomycin (8). *Treponema* growth was inspected after 24, 48, and 72 hours of incubation at 42° C in a BBL anaerobic system with catalyst and in a microaerophilic atmosphere (12).

RESULTS

Clinical observations

The pups had rhinitis and conjunctivitis. They were vomiting mucus with small amounts of food. Profuse watery diarrhoea with small amounts of blood was seen. Two pups were coughing and pneumonia was diagnosed. Blood and serum analysis revealed hyppoproteinaemia (35 g/l mean value), hypoglycemia (15 mmol/l), anaemia (3.9 10^{12} erythrocytes) and uraemia (3.50 mmol/l). The dogs with pneumonia showed lymphocytosis.

Pathological findings

The gastro-intestinal wall was swollen and congested. The turgor of the intestinal loops was increased. In the stomach there was abundant mucus. A catarrhal enterocolitis with watery contents was seen.

The caecum and colon contained some fibrinous material mixed with a little blood. The mesenteric lymph nodes were swollen and congested.

Spirochaetes were observed on Giemsa stained smears of the gastric mucus from the fundus and the pylorus.

Histologically, the villi of the terminal jejunum were atrophic in only one dog. Some of the crypts of the small intestine were dilated and filled with cellular debris. Deposition of cell debris was also observed in numerous crypts of the caecum and colon.

Two dogs had purulent rhinitis and severe catarrhal bronchopneumonia of the ventral parts of the apical and cardial lobes. Hepatocytes showed cloudy swelling and vacuolar degeneration. Tubulonephrosis was seen. The spleen was slightly swollen and depleted, with activation of the germinative centres.

Electron-microscopical inspections of the middle jejunal epithelium revealed no significant lesions. In the colon ultrastructural epithelial cell lesions were found mainly around the mouth of the crypts. A number of absorptive epithelial cells were in a stage of degenerative change (Fig. 1). The microvilli were short and irregular, or sometimes even completely absent. The number of free ribosomes had increased. The cell nuclei were often swollen, with a patchy condensation of the chromatin, which was mainly lining the nuclear envelope. Mitochondria were swollen. The cytoplasm of the affected cells had become either clear or darkened. The intercellular junctions had sometimes lost contact. In a more advanced stage of degeneration, the cells were rounded, and subsequently they were released into the intestinal lumen. In the majority of these degenerating epithelial cells, intensely staining corona virus-like particles were seen (Fig. 2).

They were present in the cytoplasm inside vacuoles. The particles were moderately pleomorphic, ranging from 50 to 90 nm in diameter. They consisted of a nucleocapsid of 40 to 80 nm, separated from an outer unit membrane by an interlacing space of 2 to 6 nm. In the centre, an electron translucent centre could often be seen with a diameter ranging from 0 to 50 nm. The particles were sometimes seen in great numbers inside large cytoplasmic vesicles, at other times they were single particles each embedded in a small vesicle (Fig. 2). Viral particles were sometimes present between the la-



Fig. 1. Colonic epithelial cell degeneration. 1. Epithelial cell release into the intestinal lumen. 2. Short, irregular microvilli. 3. Patchy condensation of chromatin, lining the nuclear envelope. 4. Cleared cytoplasm. 5. Intracytoplasmic lipid droplets.
EM x 1400, photographic
} enlargement
} magnification x 1.2

mellae of the endoplasmic reticulum. Similar particles were sometimes observed inside the Golgi apparatus. Occasional bud like structures were observed at membranes of cytoplasmic vacuoles or dilated cisternae of the endoplasmic reticulum. No budding of viral particles from the cell membrane of the infected cells was seen. Small, single viral particles embedded in vesicles were often seen in the apical part of the cytoplasm, near the cell membrane. Viral particles were also seen in large numbers between the microvilli of heavily infected cells (Fig. 3). The nucleus did not contain viral particles on any occasion, nor did the goblet cells. A number of epithelial cells lining the intestinal lumen contained large lipid droplets and vacuolarly distended endoplasmic reticulum (Fig. 1). These lipid-containing

cells did not always show up in viral particles.

Virology

Electron-microscopical examination of the intestinal contents of a pup having diarrhoea for at least one day revealed the presence of particles with a typical coronavirus morphology (Fig. 4). The particles were pleomorphic with a mean diameter of 107 nm including the projections. Rotaviruses or parvoviruses were not observed.

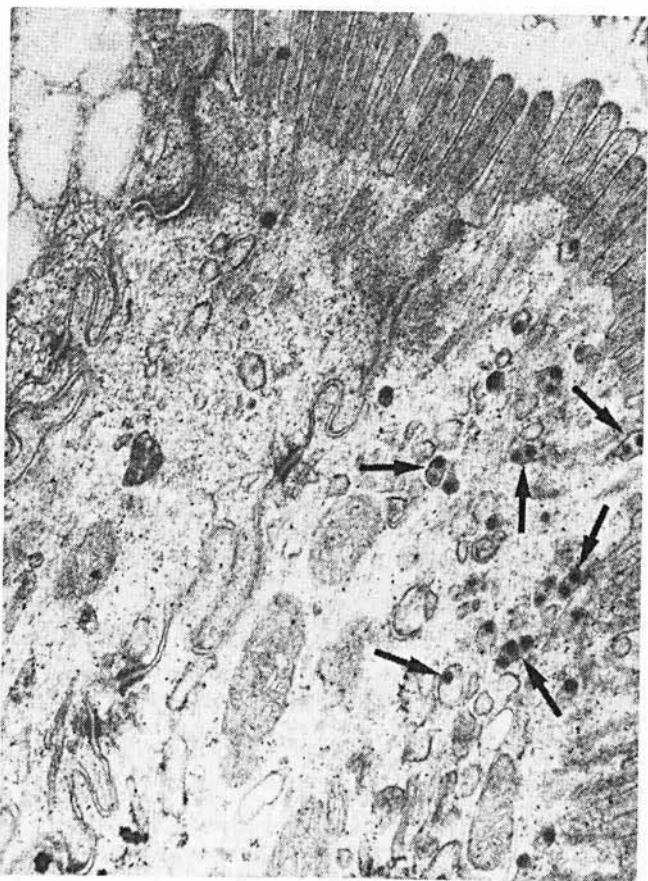
Bacteriology

Salmonellae were not isolated. *Clostridium perfringens* was found in three pups. Non-haemolytic treponemata were isolated on plates incubated in either a microaerophilic or in an anaerobic en-

Fig 2. Enterocyte, containing single virus-like particles inside small cytoplasmic vacuoles (arrows).

EM x 9500, photographic

{ enlargement
{ magnification x 1.2



environment with and without polymyxine. *Campylobacter jejuni* was demonstrated in all parts of the intestines of the poodle pups and in the faeces of the five other dogs with loose stools.

DISCUSSION

The morphological and morphogenetical properties of the particles observed in the large intestinal contents and in the colonic epithelial cells are characteristic of coronaviruses (14, 20). The observed virus particles are morphologically similar to the canine coronavirus described by Binn *et al* (3). This virus causes diarrhoea in dogs (7, 19).

Multiplication of canine coronavirus in colonic epithelial cells has not previously been reported. Transmissible gastroenteritis (TGE) virus, a coronavirus of pigs, does not affect the colon (16).

However, infection of colonic epithelial cells by coronavirus has been demonstrated for Neonatal Calf Diarrhoea Coronavirus (NCDCV) (15, 17). In this sense, the present coronavirus would be more similar to NCDCV than to TGE, although a number of authors claim that the canine coronavirus is serologically related to TGE, if not identical with it (1, 10, 13). The literature (7) as well as research data seem to suggest that the canine coronavirus infection proceeds in the caudal direction over a certain period of time.

The presence of virus particles inside the Golgi apparatus has been reported for canine coronavirus as well as for other coronaviruses (16, 20). The significance of this in the process of virus maturation is not clear. The interaction of virus and intestinal cells, and the resulting absorptive cell degeneration, suggest that the canine coronavirus is pathogenic for the

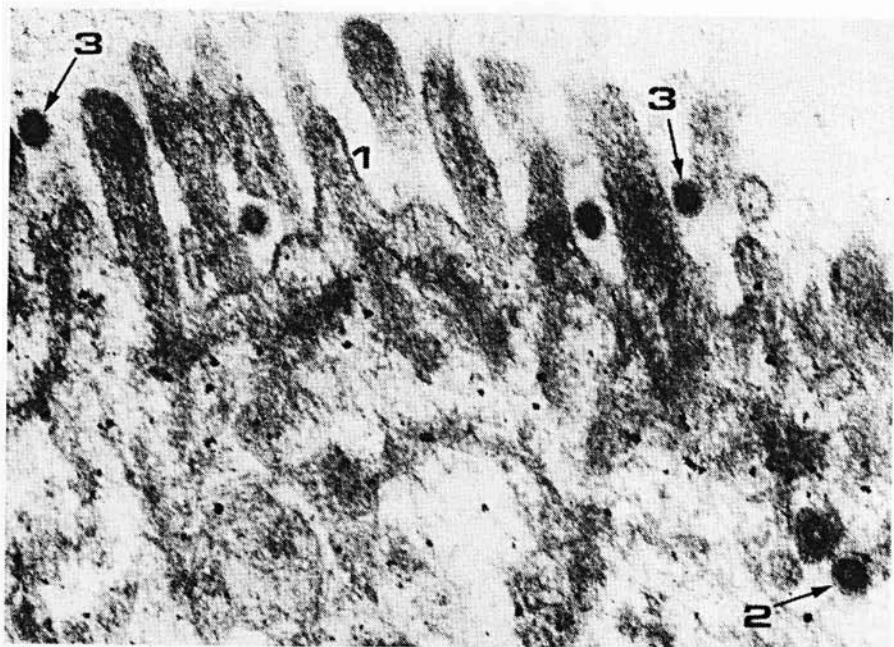


Fig. 3. Apical part of the cytoplasm of a colonic epithelial cell. 1. Short, irregular microvilli. 2. Virus-like particles embedded in small cytoplasmic vesicles. 3. Virus-like particles lying free between the microvilli.
EM x 28000, photographic enlargement x 1.2

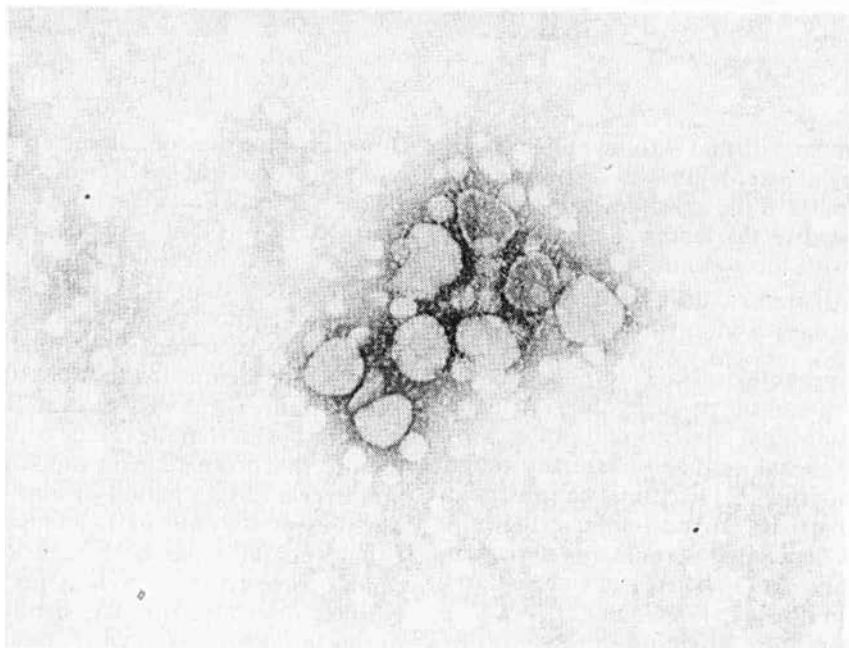


Fig. 4. Particles with a typical coronavirus morphology as found in the large intestinal contents. Negative staining PTA 2 per cent.

Em x 28,000, photographic enlargement x 3

colonic epithelial cells. Destruction of these cells means that absorption of liquids will be interfered with. This phenomenon may account for the severity of the clinical symptoms observed in these pups, especially the rapid dehydration. *Clostridium perfringens*, *Treponema innocens* (8,9), *Campylobacter jejuni* (3) and gastric spirochaetes (21, 22) are common findings in dogs. Primary pathogenicity of these bacteria in dogs has never been proved, but a secondary pat-

hogenic role is not excluded. It is suggested that the present coronavirus was the causative agent of the gastrointestinal disturbances. As a consequence the bacterial intestinal balance was disturbed and there was an increase of gastric and faecal spirochaetes and spirillae in the colon.

This intestinal disfunction was complicated by hepatic and renal degeneration and by severe rhinitis, conjunctivitis, and bronchopneumonia.

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