Canine Circovirus Enteritis

H. J. Van Kruiningen¹, Mizuki Heishima, Kirklyn Kerr, Antonio Garmendia, Zeinab Helal and Joan Smyth

Connecticut Veterinary Medical Diagnostic Laboratory
Department of Pathobiology and Veterinary Science
University of Connecticut, Storrs, CT 06269-3089

¹Corresponding author: H. J. Van Kruiningen, Department of Pathobiology and Veterinary Science, 61 North Eagleville Road Unit 3089, University of Connecticut, Storrs, Connecticut 06269-3089. Herbert.Vankruiningen@uconn.edu

Abstract

A 5-month-old Bassett/ Labrador Retriever cross was autopsied following a bout of lethargy, inappetance, and bleeding gums. Mucous membranes were white and small intestines blue-black; colon contained black feces. The spleen was swollen and multiple lymph nodes were enlarged and hemorrhagic. Microscopically, intestine had focal crypt cell necrosis and focal circumferential vasculitis, the latter the cause of the blue-black coloration. Spleen and lymph nodes had necrosis of lymphocytes, and some nodes had erythrophagocytosis. Vasculitis and associated inflammation occurred in brain, meninges, lung, liver and kidneys as well. Electron microscopy revealed aggregates of 15-18 nm round virus particles in damaged crypt cells and in endothelium of small blood vessels. Electron dense intracytoplasmic inclusions consisting of paracrystaline-arrayed virus were demonstrated in macrophages in lymph nodes. These virions and inclusions were identified as circovirus. Real time PCR confirmed the identity. This case represents the first instance of canine circovirus enteritis recognized outside the state of California.
In 2013 Li et al described a very dramatic severe hemorrhagic gastroenteritis and multisystemic vasculitis in a young dog\(^1\). They characterized the complete genome of a canine circovirus (DogCV) responsible for the lesions and then retrospectively found three additional cases in material retrieved from the Veterinary Medical Teaching Hospital at the University of California, Davis. A year later Decaro et al characterized a related circovirus recovered from hemorrhagic enteritis in a young dog in Italy\(^2\). Here we report the first US case recognized outside the state of California.

In late September 2017 a 5-month-old black and tan castrated male Bassett/Labrador Retriever dog was brought for autopsy, approximately 12 hours after its death. This dog and a female littermate had been rescued two months earlier and held at a shelter, having originated in Mississippi and having been transferred to Connecticut via a rescue service in San Antonio, Texas. The dogs had been vaccinated against canine distemper, hepatitis, parainfluenza, parvovirus, coronavirus, leptospirosis, \textit{Bordetella} and rabies at both facilities. Castration was done in Texas.

Two days prior to death this dog had lethargy, inappetance and bleeding gums. The day before its death it ate a small amount of dog food, drank excessive water, “looked sick” and became recumbent. At 4 AM on the morning of the third day the dog was heard to make an agonal sound, and died. Eight days after the death of this dog its littermate became ill with diarrhea, thrombocytopenia and splenomegaly. That dog was treated for ehrlichiosis and recovered.

At the time of autopsy dog number one weighed 9 kg and appeared in good body condition, neither obese nor thin, nor dehydrated, however the oral mucosa and conjunctiva were pale white. The oral cavity and entrance to esophagus and trachea contained brown ingesta, perhaps from agonal vomiting. In the chest, the lungs were mottled dark red, red, pink and gray, with some emphysema of one lobe. The major findings occurred in the abdomen, where the small intestine was striking, distended and blue in color (Fig 1). The bluish coloration appeared in a variegated pattern, created by numerous oval dark maroon to blue patches with intervening normal off-white tissue (Fig 2). The colon was bluish gray.

When the gastrointestinal tract was opened, a dramatic color change was apparent, the lesser curve of the stomach and torus pyloricus being their usual off-white, lightly
speckled with petechiae (or focal ulcers) and the antrum white, whereas mucosa of the duodenum and remainder of the small intestine was maroon to black in color (Fig 2). Mucosal ridges of the colon were streaked with black petechial hemorrhages and feces were black.

The liver was partially discolored by pseudomelenosis and had multiple flat pinpoint foci in the surface parenchyma. The spleen was swollen with rounded edges, dark red to maroon in color. Lymph nodes throughout the body were enlarged and had hemorrhagic cortices; these included submandibular, cervical, tracheobronchial, hepatic, mesenteric and internal iliac nodes.

Representative tissues were fixed in 10% formalin, processed in graded alcohols and xylene, embedded in paraffin, cut at 4 microns thickness and stained with hematoxylin and eosin. Other 4 micron sections were subjected to immunohistochemistry for canine parvovirus, coronavirus and canine distemper. Other stains included Lendrum’s phloxine-tartrazine and Shorr’s Page Green for viral inclusion bodies, Twort’s Gram stain for bacteria, trichrome, and phosphotungstic acid hematoxylin.

For electron microscopy tissue samples were cut into 1 mm² blocks and placed into 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. Secondary fixation was done in 1% osmium tetroxide. Tissue was dehydrated through a series of graded alcohols and embedded in Glauert resin containing Embed-812. Ultrathin (~ 70 nm) sections were cut with a diamond knife, decompressed with trichloroethylene, and collected on 100 mesh copper grids. Sections were counterstained with 2% aqueous uranyl acetate, then 2.5% Sato’s lead citrate. Images were obtained using a bright field FEI Tecnai Biotwin G2 Spirit transmission electron microscope.

Microscopically, the major lesions in the gastrointestinal tract were limited to small intestine. There were foci of crypt cell necrosis that attracted attention (Fig 3 and 4). These were scattered randomly around the circumference in every part of the small bowel. Additionally, there was focal hemorrhage of the deep lamina propria, just above the muscularis mucosae and at mid-level submucosa. These foci of hemorrhage were centered on small vessels and did not coincide with the distribution of crypt cell necrosis. The vascular changes consisted of swelling and slough of endothelial cells, disrupting the continuity of the endothelium. Often only three or four rounded cells remained through the circumference, leaving bare basement membrane. The vasculitis was focally distributed throughout small intestine and only rarely accompanied by thrombi (Fig 5). Fibrinoid necrosis of the media of some of the small vessels was only occasionally discernable. The villi and lymphatics of the intestine were unaffected.

The stomach had only focal mucosal hemorrhage, whereas the ileum, cecum, and colon had necrosis of lymphocytes at the centers of submucosal lymphoid follicles, including
those of Peyer’s patches. There was hemorrhage at the centers of some of the
damaged lymphoid follicles. The colon had some focal vasculitis and hemorrhage as
well, but much less severe than that of the small intestine.

Severe, but focal vasculitis occurred in brain, meninges, myocardium, lung, liver, and
kidney (Fig 6 and 7). In the liver, the distribution was both central and periportal as well,
creating a variable pattern of necrosis. In the kidney, the cortex was affected. In each of
these tissues, vessels with vasculitis were accompanied by mononuclear cell
perivascular inflammation, often eccentric, and consisting of plasma cells and small
histiocytes. In the meninges, this constituted a mononuclear cell meningitis. In the
spleen and lymph nodes, there was marked lymphocyte necrosis, hemorrhagic in the
latter, some accompanied by erythrophagocytosis. The nodes had marked sinu
histiocytosis; and the bone marrow lacked megakaryocytes.

At the electron microscopic level, enlarged damaged crypt cells with granular, globular
contents were found to contain variably sized aggregates of 15 - 18 nm round virus
particles Fig 8) These were sometimes arranged in rows (Fig 9) The virions were
uniform in size with dense centers and lighter collars. Inclusion bodies were rare (Fig
10). Endothelial cells of affected vessels were rounded (Fig 11) or sloughed.
Electronmicroscopy of macrophages of affected lymph nodes identified electron dense
inclusions composed of virus particles arranged in paracrystaline arrays (Fig 12 and
13).

Immunohistochemistry for parvovirus, coronavirus, and canine distemper virus was
negative. The Lendrum and Shorr’s stains for virus inclusions were disappointing, only
rarely disclosing what might have been seen as round, intracytoplasmic inclusions. The
Gram stain revealed postmortem bacteria in some tissues. Trichrome and PTAH
revealed only rare thrombi in damaged vessels and did not enhance the appreciation for
fibrinoid necrosis of the media.

To identify canine circovirus (CACV) in tissues a Taq man real time PCR reaction was
performed essentially following methods and using primers and probe described
previously (Li et al 2013). To this end, DNA was extracted from intestine, liver and
spleen using the DNAeasy extraction kit following the manufacturer’s instructions
(Qiagen, Germantown, MD). The PCR reaction mixture contained Taq Man Fast
Advanced Master Mix (Applied Biosystems, Austin Texas), forward primer 5’-
CTGTTGTGAAACTGAAAGAGACGA-3’, reverse primer 5’-
TGACGTAGGTCTCCGATACG-3’, FAM-AGCCTTGCCGCTGTCGTC-GTACGTCGTC-BHQ1 probe
for CACV-2 capsid, DNA template and nuclease-free water. The reaction was run in a
BioRad CFX96 C1000 Real Time System (BioRad Hercules, CA) at 95°C for 5 minutes,
followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The DNA
extracted from the three tissues being tested yielded positive reactions in Taq Man real
time PCR to different extents. Additionally, the DNA extracted from the spleen was also
amplified with the same CACV-2 capsid primers by conventional PCR and the amplicon
was run and extracted from agarose gel and subjected to Sanger’s sequencing. The
sequence thus obtained confirmed further the identity of the virus as canine circovirus and matched closely the CACV strain OH19098-1 (GenBank: MF457592.1).

This constitutes the first case of canine circovirus disease outside of California. Our findings raise the questions: where did the virus originate, in Mississippi, in Texas, or in Connecticut and why does this appear to originate in shelter dogs? We will have to wait for additional cases to get the answer to these questions and to know the significance.

This virus causes the most severe enteric disease these authors have seen in canines. The crypt cell necrosis is unique and reminiscent of coronavirus crypt damage in winter dysentery of cattle and coronavirus enteritis of calves. The vasculitis effect of pooled deoxygenated blood results in a blue discoloration of the intestine – a remarkable intestine, in this canine and in porcine circovirus type-2 (PCV-2) disease of swine. At the time this dog was recumbent and while the owner was concerned about the oral hemorrhage, she reported that the dog had the odor of a “GI bleed” She, being a nurse, had recognized the characteristic odor, a finding consistent with the appearance of the intestinal contents shown in Figure 3. A similar odor of a GI bleed occurs in winter dysentery of cattle, where the feces are similar. The gastrointestinal elements of this disease belie the very severe damage that occurs as a consequence of virus-induced vasculitis in many other organ systems. One wonders if there is a human counterpart.

The viruses and viral inclusion bodies we demonstrate here are identical to those described and shown by Li et al in the California dog and to those of porcine circovirus (PCV) in infected PK-15 cells and in the lymph nodes of pigs with postweaning multisystemic wasting syndrome.

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References


Legends for Figures

Fig 1. Stomach and pancreas are unaffected; entire small intestine is discolored dark red to blue from numerous oval red to black infarcts. Colon contents are maroon colored, a mixture of blood and feces.

Fig 2. Opened small intestine reveals numerous oval dark red to black infarcts.

Fig 3. Intestine. Focus of crypt cell necrosis. Hematoxylin and eosin, 600X

Fig 4. Intestine. Similar focus stained by the Lendrum method. 600X

Fig 5. Small bowel submucosa containing a thrombosed vessel and surrounding hemorrhage. Lendrum, 400X

Fig 6. Cerebrum; vasculitis. Few intact endothelial cells remain, replaced by mononuclear leukocytes. H & E, 400X
Fig 7. Cerebrum. Similar vasculitis, revealing mononuclear leukocytes and a few large cells with eosinophilic cytoplasm (ballooned endothelial cells or macrophages). H & E, 400X

Fig 8. Electron micrograph of virus particles in the cytoplasm of a crypt epithelial cell. 98,000X

Fig 9. Aggregates of virus, some arranged in rows, in a crypt epithelial cell. 98,000X

Fig 10. Intracytoplasmic inclusion body in a crypt epithelial cell. 68,000X

Fig 11. Ballooned nucleus in a vascular endothelial cell from affected intestine. 13,000X

Fig 12. Circovirus in paracrystalline array in macrophage cytoplasm from an affected lymph node. 98,000X

Fig 13. Paracrystalline arrayed circovirus in macrophage cytoplasm from affected lymph node. 98,000X