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Fatal coronavirus infection in puppies following canine parvovirus 2b infection

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Canine coronavirus (CCV) belongs to the Coronaviridae family, which includes enveloped and pleomorphic viruses 60–220 nm in diameter, helical nucleocapsids, and petal (club-shaped) peplomeres, which are widely spaced on the envelope, resembling a solar corona.[1,12]

CCV was first isolated in 1971 from military dogs in Germany suffering from gastroenteritis.[3] Subsequently, additional cases have been reported that were usually mild and self-limiting, unless complicated by canine parvovirus (CPV) infection.[2,7,8,10,14] Coronaviral disease in dogs is extremely variable. Although adult dogs generally have no or mild clinical signs and usually recover after a brief period of illness, pups with secondary bacterial infections, parasites, or other viruses may suffer severe, even fatal, disease.[1,8,10] Infected dogs shed CCV in the feces for 6–9 days, but shedding can be prolonged in some animals.[1,9] The most likely mode of transmission is by the fecal–oral route.

The main target of CCV is the small intestinal epithelium, where a lytic infection results in desquamation and shortening of duodenal and jejunal villi. Pathologic changes have been observed mainly in experimentally infected dogs and are characterized by dilated intestinal loops filled with watery ingesta and feces, congested or edematous mucosa, and edematous mesenteric lymph nodes.[1,8,9,13,14] Microscopic changes are characterized by atrophy and fusion of intestinal villi, a deepening of crypts, and increased cellularity of the lamina propria.[8,9]

In this report, we describe the clinical, virologic, and histopathologic findings observed in littermate pups that died with signs of severe hemorrhagic enteritis 15 days following recovery from a CPV-2b infection. The presence of parvovirus-like intranuclear inclusions in intestinal epithelial cells >2 weeks following recovery from CPV-2b infection and the presence of pulmonary lesions were unexpected, and the CCV infection may have been complicated by infection with another agent such as the minute virus of canines (MVC, canine parvovirus 1).

In the initial disease episode, 4/4 Corso (Italian breed) pups, 45 days old, developed hemorrhagic gastroenteritis. CPV. 2b was the only virus detected by laboratory examination, which included hemagglutination (HA) tests on fecal suspensions, viral isolation attempts in A-72 cell cultures, indirect fluorescent antibody tests (IFAT), and antigenic characterization of isolates with monoclonal antibodies. All 4 pups recovered uneventfully and appeared normal after 1 week.

Fifteen days after the initial outbreak, 3 of the 4 pups (60 days old) again had signs of hemorrhagic gastroenteritis, but signs were much more severe than those in the first episode. Two pups died within 3 days after the onset of clinical signs, and the third pup recovered.

Gross pathologic examination of the 2 dead pups revealed severe necrotizing hemorrhagic enteritis involving the entire small intestine, and emphysematous foci were scattered over the hyperemic and edematous lungs. Examination for parasites was negative, and bacteriologic cultures did not reveal any significant bacterial pathogens.

HA tests were performed on chloroform-treated fecal samples, using 1% swine erythrocytes at 4 C, as previously described.[5] HA tests also were performed on the supernatant fluids of A-72 cell cultures that had been inoculated with the fecal samples.

Fecal samples, treated as described above, were inoculated into A-72 canine cell cultures grown in Dulbecco's minimal essential medium that contained 10% fetal bovine serum. After 4 days of incubation, if cytopathic effects were absent the cells were frozen and thawed 3 times and additional passages were made with each cryolysate. Inoculated A-72 cell cultures also were fixed in acetone at 2-day intervals and stained by the IFAT using CCV monoclonal antibodies and a monospecific CPV-2 canine antiserum.

Samples of stomach, small intestine, cecum, colon, rectum, mesenteric lymph nodes, spleen, liver, kidneys, and lung obtained from the 2 animals that died were fixed in 10% buffered formalin. After fixation, the tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin (HE).

All 3 fecal samples were negative for CPV-2b by the HA test. By the second passage, the cell cultures all showed a limited cytopathic effect (CPE) after 48–72 hours, characterized by rounded refractile cells unlike the CPE that occurs with CPV-2b or canine adenovirus (CAV) infections. In subsequent passages, the CPE appeared approximately 24 hours postinfection and was maximal within a few hours, with >50% of cells affected.

By the IFAT using CCV monoclonal antibodies, the A-72 cells showed typical intracytoplasmic fluorescence by 2 days postinoculation. This fluorescence was not present in uninfected cells or when CPV monospecific serum was employed.

Histologic changes in the mucosa of the small intestine were characterized by atrophy and flattening of most villi.

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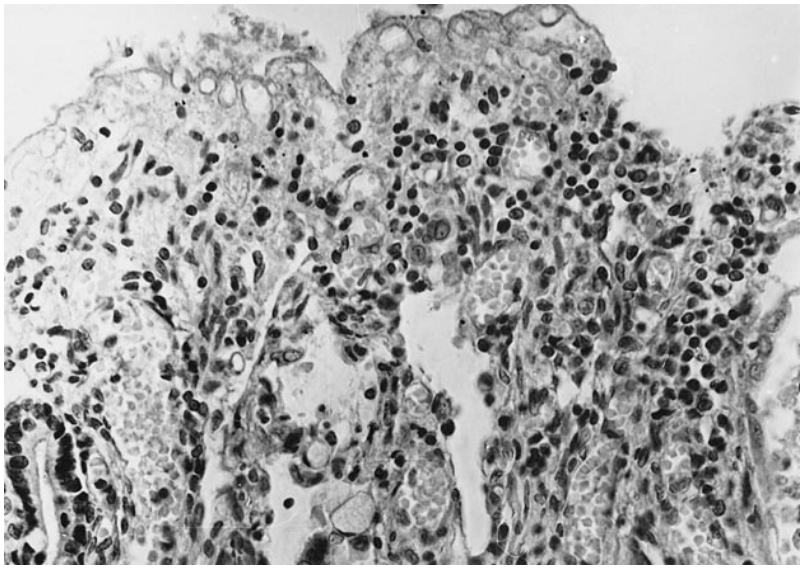

Figure 1. Ileum dog. Atrophy and flattening of villi, with crypts becoming more superficial. HE, 290X.

Intestinal crypts were often dilated and contained increased numbers of goblet cells. The lining epithelium was sometimes focally denuded (Fig. 1). Club-shaped and fused villi also were common. A mononuclear infiltrate was generally present in the lamina propria.

Regenerative hyperplasia of the epithelium was present in some crypts. Regenerative changes consisted of tall hyperchromatic cells containing nuclei with prominent nucleoli and a moderate number of mitotic cells. Parvovirus-like intranuclear inclusion bodies were observed in some intact crypt epithelial cells (Fig. 2). Necrotic areas also were observed in the jejunal mucosa of one of the pups. Spleen, mesenteric lymph nodes, and Peyer's patches were severely depleted, and there was necrosis of lymphoid cells. In the spleen particularly, lymphoid elements appeared to be dramatically reduced in both the periarteriolar sheaths and lymphoid follicles, where karyorrhexis was evident (Fig. 3).

The clinical evolution of the CCV infections described here was particularly severe, and 2 of the 3 infected pups died. This finding is in contrast with what is normally observed during CCV infections, where the clinical course is generally mild.[1,3,8,9] The histologic lesions observed in the 2 dogs studied appeared to be the consequence of the CCV infection that followed recovery from the CPV-2 infection 15 days earlier. Alterations of the intestinal villi were typical of CCV infections[1,8,9] however, basophilic intranuclear inclusion bodies in crypt epithelial cells, as well as necrosis

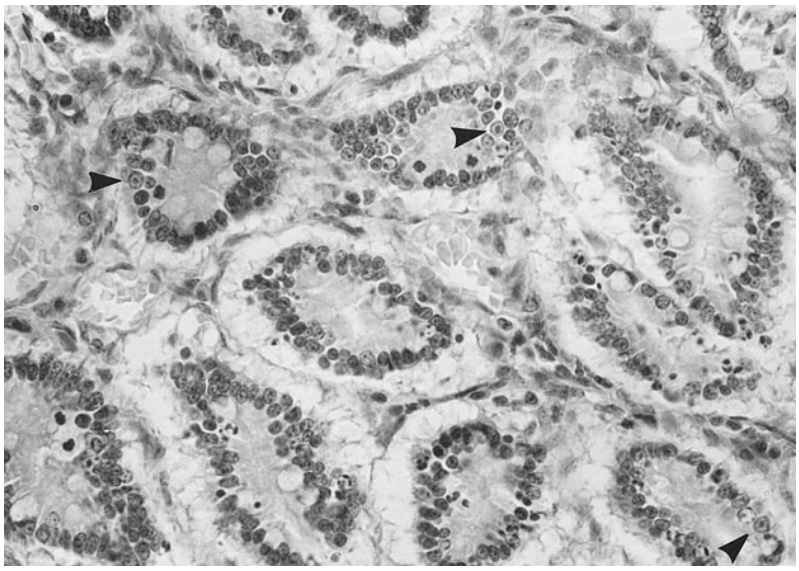

Figure 2. Ileum, dog. Intranuclear inclusion bodies are in epithelial cells of crypts (arrows); moderate mitotic activity is evident in the crypt mucosa. HE, 550X.

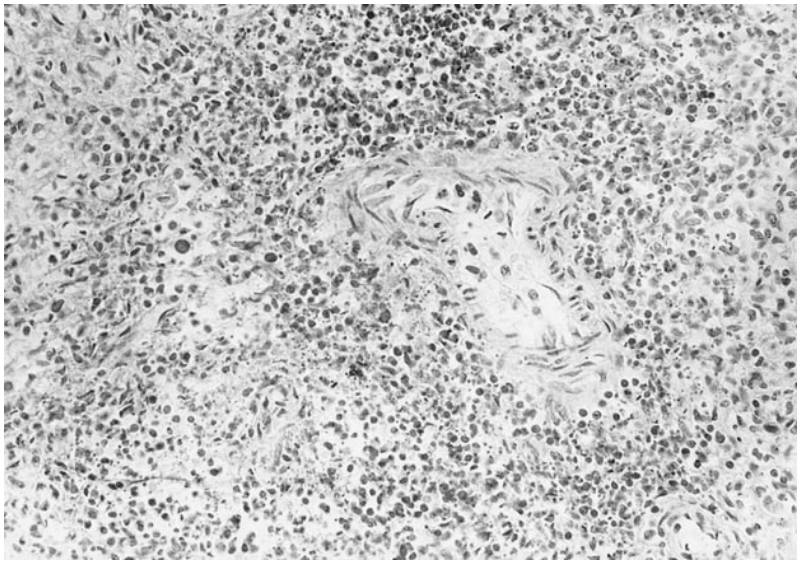

Figure 3. Spleen, dog. Necrosis of lymphocytes in lymphoid follicles. HE, 350X.

and depletion of lymphoid tissue, are characteristic findings in CPV but not CCV infections.[4,11] Those findings notwithstanding, cells still infected by CPV-2b would not be expected to persist 3 weeks after the initial infection,[11] and CPV-2b was not demonstrated in the pups' feces by HA or the IFAT. Furthermore, experimental studies on CPV-2b pathogenesis in pups failed to reveal virus, or viral DNA in tissues >6 days after oronasal infection by virus isolation or in situ DNA hybridization methods (D. Peters, unpublished data). CAVs would likely have been isolated with the cell cultures used if they had been present in the fecal samples. These findings suggest the intriguing possibility of the involvement of another virus, perhaps the MVC.[6] This question is currently being investigated; in June 1998, MVC was isolated from young puppies that died in Puglia, Italy.

Although CCV has been found in dogs concurrently with CPV-2[7,14] and infection with CCV can enhance the severity of a subsequent CPV-2 infection,[2] CPV-2b infection preceded the CCV infections by nearly 3 weeks in these pups. There are previous reports of enhancement of CCV susceptibility or disease severity by a previous CPV (2 or 2b) infection.

The previous infection by CPV-2b might have contributed to the severity of the subsequent CCV infections in these pups; however, the finding of intranuclear inclusions typical of parvoviruses approximately 2 weeks after recovery from CPV-2b infection is puzzling. CCV almost exclusively infects small intestinal villus epithelial cells,[1,3,9] whereas CPV-2b replicates only in actively dividing cells such as lymphocytes, intestinal crypt cells, and myocardial cells in newborn pups.[8,11] In dogs, the recovery period from the CCV infection is characterized by active replication of the intestinal crypt cells, leading to repair of damaged intestinal villi. Under such conditions, those cells become ample sites for parvovirus replication; however, the pups were very likely immune to CPV-2b. The parvovirus-like inclusions in the small intestinal villi and gross pulmonary lesions were similar to those described for MVC, a virus recently isolated in Italy. Thus, simultaneous infection by CCV and MVC may have resulted in the exceptionally severe enteritis that was observed, a possiblity that is now being studied.

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Dermatophilosis in captive tortoises

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Dermatophilus infections have been observed in several lizard species, a boa constrictor (*Constrictor constrictor*), and an American alligator (*Alligator mississippiensis*).[1,3,5,6,10,11,13] Lesions in reptiles have been described as surface crusts comprised of necrotic cellular debris, keratin, and inflammatory cells, with necrosis of the epidermis or caseous subcutaneous nodules. Dermatophilosis was diagnosed by isolation of *D. congolensis* in culture or identification of its unique branching, filamentous morphology in lesions.

Until recently, *D. congolensis* was the only species assigned to the genus *Dermatophilus,* and phenotypic properties of the species were said to vary considerably.[4] A new species designation, *D. chelonae,* was proposed for 3 highly divergent isolates from chelonids in Australia.[10] Chelonid isolates appear to be adapted to poikilotherms, growing better at lower temperatures than *D. congolensis* and having low infectivity for mammals.[10] There is little information available on disease caused by *D. chelonae* in reptiles and its recognition in the field. The purpose of this report is to describe the isolation of *D. chelonae*-like bacteria from cutaneous and visceral lesions in 2 species of captive tortoises.

Five bowsprit tortoises (*Chersina angulata*) and 3 Egyptian tortoises (*Testudo kleinmanni*) were housed in adjacent sections of an open tripartite wooden box. Another bowsprit tortoise was housed separately in a glass terrarium. Sphagnum moss bedding was used. Room temperature was maintained at approximately 30 C. A daily photoperiod of 12 hours was maintained with a light source.[a] Fecal matter was removed regularly, and the boxes were emptied and washed with detergent and half of the sphagnum moss was replaced monthly.

The 5 bowsprit tortoises were wild-caught. They had been housed together since their arrival in 1991. The sixth bowsprit tortoise had been housed with the group until September 1995. The 3 Egyptian tortoises were of unknown origin. They were received from 2 separate donors in 1994 and were housed in the box from September to November 1995. Several times during the fall and winter of 1995/1996, rain water leaked into the box.

In November 1995, cutaneous lesions appeared simultaneously in 3 bowsprit tortoises, including the one in the glass terrarium. Each tortoise had multiple skin lesions (>10), especially on the neck and in deep recesses around the neck and legs (Fig 1). The lesions were yellow-white, 0.2–1.2-cm-diameter nodules covered with dry flaking skin with a tract of yellowish material extending into the dermis. One tortoise was euthanized because of the extensive skin nodules and ulcerative stomatitis. Another tortoise also had bilateral septic gonitis, and a fourth bowsprit developed skin lesions and bilateral septic arthritis 5 months later.

Skin and joint lesions were debrided and cleansed with dilute povidone–iodine solution. In addition, parenteral and oral antibiotics (ampicillin and amikacin or enrofloxacin, and metronidazole) were administered. The skin lesions regressed in 3–8 months but recurred at different sites within 12 months.

Two bowsprit tortoises and 1 Egyptian tortoise were found dead with no antemortem signs between January and April 1996. Tissues from these and the euthanized bowsprit were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE), Gram's, Grocott's methanamine silver (GMS), and Fite–Faraco acid-fast procedures.

Skin biopsies from 2 bowsprit tortoises were examined histologically. Both samples had intracorneal accumulations of heterophils with bacteria. Heterophils at the periphery had undergone coagulative necrosis with uniform eosinophilic staining of the nucleus and cytoplasm and loss of cytoplasmic granularity. The necrotic heterophils formed poorly defined layers, divided by bands of keratinocytes (Fig. 2A). The epidermis at the base of the lesion was mildly acanthotic with numerous transmigrating heterophils. The subjacent dermis contained increased fibroblasts, a moderate infiltrate of heterophils, and a few lymphocytes (Fig. 2B). The subcutis had a mild to moderate heterophilic infiltrate, perivascular lymphoid infiltrates, and myxomatous metaplasia. Organisms were not visible with HE, but a Gram stain revealed gram-variable branching filamentous bacteria of varying width within the exudate (Fig. 3). The bacteria were not acid fast with Fite-Faraco but stained with GMS.

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