

phe obsoleta quadrivittata) was treated with radiation therapy, which delayed local recurrence.¹¹ Cobalt therapy was unsuccessful in the treatment of a lymphosarcoma in an Indian python (*Python molurus*) and an angiosarcoma in a spitting cobra (*Naja nigrocollis*).^{7,11} Chemotherapy has been used rarely in reptiles.⁸ Cytosine arabinoside has been unsuccessfully used in the treatment of a lymphosarcoma in a rhinoceros viper.⁹ In this kingsnake, chemotherapy was not selected because the tumor appeared localized and without metastases.

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Typhlocolitis caused by *Clostridium difficile* in suckling piglets

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Clostridium difficile, a gram-positive to gram-variable, spore-forming, anaerobic bacterium, is a common inhabitant of soil, water, and the intestinal tract of various mammals, birds, and reptiles.¹⁶ It has been identified as a cause of pseudomembranous colitis in humans,^{2,4,21,24,27} enterocolitis in foals,¹⁶ nosocomial diarrhea and typhlocolitis in adult horses,^{20,22} typhilitis in adult hamsters,^{3,7} and cytopathic toxin production in guinea pigs treated with penicillin.² Spores are heat resistant, can survive for months or years, and are carried readily on hands and equipment.²¹ Recovery of *C. difficile*, in association with *Balantidium coli* and *Salmonella*

typhimurium, from 8-week-old pigs with a naturally occurring enterocolitis has been reported.¹⁵ Here, we describe a herd problem of mesocolonic edema and typhlocolitis in suckling piglets from which *C. difficile* was identified as the putative etiology.

An unusual outbreak of disease began in a newly established herd of 600 gilts 3 weeks after the first litters were farrowed. Initially, piglets were affected at about 2 weeks of age. Soon, newborn piglets also became ill. An average of 25 litters were farrowed weekly, with the number of live births per litter ranging from 9.9 to 10.3 piglets. Weekly preweaning mortality prior to the outbreak and subsequent to its resolution ranged from 7% to 9%. During the several months in which clinical signs of disease were noted, weekly losses from all causes ranged from 7% to 58% (\bar{x} = 25%). The practitioner estimated that the disease described here accounted for at least 90% of the losses, but necropsies were

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Figure 1. Four-day-old piglet. Hydrothorax, ascites, and edema of ascending mesocolon. Lung is slightly atelectatic. Stomach is distended with milk curd.

not performed in the majority of cases. An estimate of morbidity was not made.

Clinical signs included dyspnea, increased prominence of ribs and vertebrae, mild abdominal distension, and scrotal edema. There were 8 separate submissions to the diagnostic pathology laboratory of the Western College of Veterinary Medicine (Saskatoon, SK) during a 6-month period. Thirty piglets, of which 21 were live and ranged in age from 1 to 14 days (\bar{x} = 5 days) were examined. Ascites, >50 ml of fluid, was present in 22 pigs. Prominent edema of the ascending mesocolon (Fig. 1) was present in 27 piglets. Hydrothorax of variable severity and precipitation of urates in renal papillae were seen in one-third of the cases. Stomachs contained large amounts of milk curd. Small intestines were empty or contained slight amounts of ingesta. Cecum and colon were flaccid and dilated, containing soft to semifluid yellowish brown feces. Diarrhea was an inconsistent finding. All of 3 1-day-old piglets with mild mesocolonic edema had striking hypoalbuminemia (10 g/liter; reference range, 20–30 g/liter), increased total serum protein (70 g/liter; reference range, 34–60 g/liter), and a decreased ratio of albumin to globulins (0.17; reference range, 0.60–1.50). Serum chemistry was not analyzed in any other pigs.

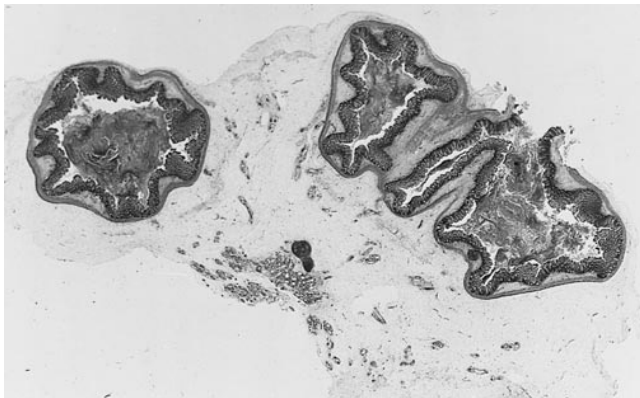


Figure 2. Two-week-old piglet. Severe edema of ascending mesocolon. HE.



Figure 3. Four-day-old piglet. Mesocolonic and submucosal edema. Exfoliation of enterocytes and exudation of mucus and fibrin and dense aggregates of neutrophils into the colonic lumen. HE.

Microscopically, sections of ascending colon had severe submucosal and mesocolonic edema (Fig. 2). Exudation of mucus, fibrin, and dense aggregates of neutrophils into the lumen occurred in multiple foci (Figs. 3, 4). Neutrophils focally concentrated in and around medium-sized veins, and neutrophils and macrophages were diffusely dispersed in the edema fluid. Mucosal lesions ranged from occasional single-cell necrosis and exfoliation to segmental, transmural necrosis of the cecum and colon. Changes in the small intestines were negligible.

Bacteria isolated from intestines by routine aerobic and anaerobic incubation included both hemolytic and nonhemolytic *Escherichia coli*, *Streptococcus*, anaerobic gram-positive cocci, *Clostridium*, *Lactobacillus*, and occasional *Proteus mirabilis*. Anaerobes, notably *C. perfringens*, were cultured in high numbers. In a previous report, epizootic diarrhea in neonatal pigs with slight edema of colonic mesenteries was attributed to *C. perfringens* type A enterotoxin.⁸ *Clostridium perfringens* enterotoxins from our cultures were not identified by mouse inoculation tests. Cytotoxic effects in cell culture^a were identified when a section of colon from 1 pig was tested for *C. difficile* toxins. Samples from each of 4 pigs in a second group were paired. High concentrations

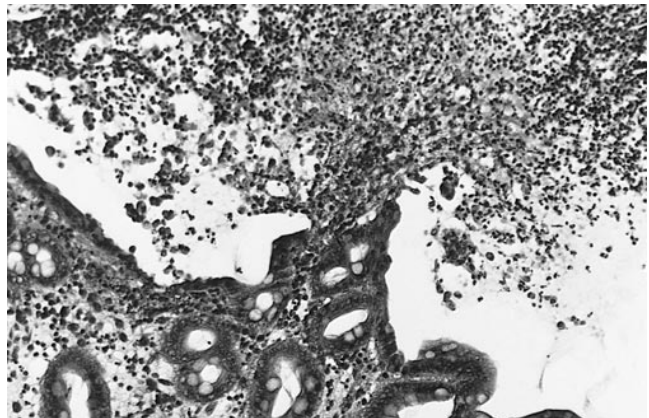


Figure 4. Four-day-old piglet. Exfoliation of enterocytes and exudation of mucus, fibrin and neutrophils into the colonic lumen. Higher magnification of Fig. 3.

of *C. difficile* toxins were found in all 4 samples by both cytotoxin assay^a and enzyme immunoassay.^b In subsequent submissions, *C. difficile* was cultured from colonic contents when specific techniques for its isolation were used.¹

Clostridium difficile is noninvasive and must colonize the cecum or colon to cause disease. When toxins are released and subsequently attach to receptors on enterocytes, lesions occur.^{2,4,12,21,27} Colonization by *C. difficile* usually is prevented by an established colonic microflora. If the normal flora is disrupted, however, the likelihood that *C. difficile* will multiply and produce toxins is increased. For this reason, infections often are associated with prior or concomitant antibiotic administration, and many occur in nosocomial environments. Infections do occur, however, without antibiotic exposure.² Other predisposing factors include stress, experimental manipulations, diet, and high environmental contamination with *C. difficile* spores.³

Numerous strains of the organism exist and differ in degrees of pathogenicity. We did not attempt to identify the strains of *C. difficile* in these pigs. In humans and horses, nosocomial diarrhea has been associated with simultaneous colonization by multiple strains of *C. difficile*, with no clonal source or point outbreak identified.^{20,26} Virulence factors include pilus expression and mucosal adherence, capsule production, and production of degradative enzymes (e.g., collagenase) in connective tissue, but the most important factor is the ability to produce toxins. Nontoxicogenic strains are avirulent.⁴

The major exotoxins produced by *C. difficile*, proteins that are the largest bacterial toxins known to date,²⁸ are toxin A and toxin B. In numerous investigations,^{5,6,13,17–19,23,28} their mechanisms of action have been more clearly defined, summarized and simplified as follows. Toxin A is called an enterotoxin because it causes fluid accumulation in the intestine. Toxin B, a cytotoxin, is extremely cytopathic for tissue-cultured cells but does not cause fluid accumulation in vivo. Toxin A is thought to initiate most, if not all, of the lesions identified in clinical disease² but toxin B could have a greater effect than previously indicated.²³ Both toxins bind to cell receptors, are internalized, and disrupt the microfilament cytoskeleton, but they do so by slightly different intracellular routing. Depolymerization of the cytoskeleton occurs, protein synthesis and cell division are inhibited, and ultimately enterocytes are exfoliated. Toxin B causes similar cytoskeletal damage in mononuclear phagocytes, but they remain viable and produce cytokines.²⁸ Indirect damage is induced by stimulation of intestinal neurons leading to degranulation of mucosal mast cells.⁵ The release of inflammatory mediators causes a marked influx of granulocytes and results in substantial tissue damage. Toxin A also induces endothelial retraction, allowing extravasation of albumin, other plasma proteins, and fluid.²⁸

Although serum chemistry evaluations were performed on only 3 pigs, the hypoalbuminemia identified is consistent with reported effects of toxin A. Leakage of albumin into tissue spaces simultaneously decreases plasma colloidal osmotic pressure and increases tissue colloidal osmotic pressure, explaining the hydrothorax, ascites, and edema seen in these pigs. Precipitation of urates in the renal collecting tubules correlates with dehydration resulting from loss of fluid

into the interstitium and gut lumen. Levels of total protein were increased despite the hypoalbuminemia, presumably because of a combination of absorption of colostral antibodies and hemoconcentration.

There is wide variation in susceptibility to disease caused by *C. difficile* among host species and age groups within species. Small numbers of individuals in several species of animals have been identified as asymptomatic carriers and excretors of *C. difficile*.² This organism easily can colonize young animals and human infants with an incomplete flora, but it disappears as the flora matures. An estimated 50–70% of healthy human infants harbor *C. difficile*;^{13,21} they are almost always asymptomatic, despite stool cytotoxin levels equivalent to stools from symptomatic adults. Infants <6 months old are resistant to disease but become more susceptible with increasing age, possibly because of maturation of toxin receptors on neonatal enterocytes.¹³ Similarly, newborn rabbits initially lack binding sites and do not become sensitive to the biologic effects of toxin A until about 45 days of age. Infant hamsters have sufficient binding sites but appear to be insensitive to toxin A.¹³ A large population of *C. difficile* is able to become established in hamster cecum by day 4 of life but by 2 weeks of age is reduced to undetectable levels, a process that coincides with maturation of the indigenous flora and rising levels of volatile fatty acids in the cecal contents.^{12,27} Disease results in older hamsters if the colonic flora is disrupted.^{3,7,25} There is a paucity of published information about the susceptibility of swine to colonization and toxin production by *C. difficile*.

Clostridium difficile apparently was responsible for causing the lesions in the affected piglets. High concentrations of *C. difficile* toxins were identified in colonic contents of several piglets, and the organism eventually was isolated. In retrospect, fecal specimens had been handled improperly by having been frozen and thawed more than once. Freezing of the fecal specimen at –20 C is reported to cause a loss of cytotoxin activity.¹ *Clostridium difficile* can be difficult to culture (hence the name *difficile*), and identification of fecal toxins is a more rapid means of determining its presence. Culture may be a more sensitive and reliable method.²⁴ Synergism has been reported between *C. perfringens* and *C. difficile* in hares¹⁰ and may have occurred in these pigs. Enteric lesions were localized in large intestines, and vascular necrosis was not identified, consistent with reports of *C. difficile* infections in other species. The severe influx of neutrophils into the mesocolon and exudation into the colonic lumen as multiple dense clusters have been described in cases of human pseudomembranous colitis.⁹

Mesocolonic edema appears to be a unique porcine lesion, ensuing from the anatomy and physiology of the ascending colon. Despite its dramatic appearance in these piglets, we consider this lesion only to be an indicator of possible infection with *C. difficile*, not a pathognomonic finding. When inoculated into gnotobiotic pigs, enterotoxigenic *Bacteroides fragilis* causes exfoliation of large intestinal surface epithelium, edema of colonic mesentery, and influx of neutrophils into the lamina propria¹¹. Gnotobiotic pigs infected with a strain of *E. coli* O157:H7 of human origin developed marked edema containing many inflammatory cells in cecal and colonic submucosa, lamina propria, and mesentery.¹⁴

Clostridium difficile is ubiquitous but rarely causes disease. It is unclear why disease developed in these piglets, but several circumstances probably contributed to dysbiosis. When the herd was assembled, the barn was new and very clean. The gilts were purchased from a single nucleus herd of "high health status" and probably were immunologically naive. They were not allowed to mingle after arrival. They had no clinical signs of disease, but oxytetracycline^c had been used prophylactically in the feed for the first 2 months. (The piglets had received no antibiotics prior to showing signs of disease.)

Affected piglets were treated with oral and parenteral antibiotics and a commercial probiotic product, but results were unsatisfactory. The only effective preventive measure was the feedback of intestines to gestating gilts. However, as the disease would abate, material for feedback would become unavailable, and subsequent cyclic outbreaks would occur. Mortality of nursing pigs ranged from 7% to 58%. The disease continued into the second parity. When an autogenous bacterin made from formalin-killed cultures of *C. difficile* and *C. perfringens* was used to vaccinate pregnant females, and bacitracin^d (275 mg/kg) was fed beginning 2 weeks prior to farrowing to reduce fecal shedding of *Clostridium*, the problem apparently disappeared. Controlled studies were not done, but preweaning mortality declined substantially, and there has been no recurrence of clinical disease. Medication was discontinued. Replacement gilts continued to be vaccinated with the autogenous bacterin for a further several months, but that practice also was discontinued.

Pathologists in other laboratories informally have stated that they have seen similar gross lesions, usually in gnotobiotic or colostrum-deprived piglets, but have not identified the cause. Because of the inherent difficulties in testing for the presence of *C. difficile* and its toxins, the actual incidence of infection probably is much greater than is recognized currently. Further investigation is warranted to assess the role of *C. difficile* as a swine pathogen and to identify the circumstances that favor its proliferation.

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Sources and manufacturers

- a. *Clostridium difficile* toxin/antitoxin kit, TechLab, Blacksburg, VA.
- b. CytocloneTM A + B EIA, Cambridge Biotech, Worcester, MA.
- c. OxySol-110, A.P.A., Sanofi Sante Animale, Canada, Victoriaville, PQ, Canada.
- d. BMD[®] 110, A.L. Laboratories, Mississauga, ON, Canada.

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Pseudocyttoplasmic inclusions in tongue epithelium of dogs with canine parvovirus-2 infections

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Members of the genus *Parvovirus* infect a wide variety of species, causing enteritis in dogs, cats, mink, and calves and reproductive failure in swine.² The viral particles are small (18–25 nm) and unenveloped and have icosahedral symmetry and single-stranded DNA genomes.⁵ Canine parvovirus-2 (CPV-2) has a predilection for actively dividing epithelial cells, and intranuclear inclusion bodies have been observed in several tissues, including intestinal crypt epithelium,^{1,3,8} myocardial cells,⁷ and monocytes in lymphoid follicles.² In a previous study of 5 dogs and 11 cats, parvoviral-associated basophilic intranuclear inclusion bodies were demonstrated in the tongue and esophagus.⁴ No other macroscopic or histologic changes were reported in the tongues of these dogs and cats. Recently, we observed basophilic to amphophilic apparently cytoplasmic inclusion bodies in hematoxylin and eosin (HE)-stained preparations of the glossal epithelium in dogs with gross and microscopic lesions of CPV-2. We examined 11 dogs with lesions consistent with parvoviral enteritis to determine the nature of these glossal epithelial inclusions and if they could be useful in confirming the presence of CPV-2 in suspected cases.

Specimens were derived from animals submitted by veterinary practitioners to the Tifton Veterinary Diagnostic and Investigational Laboratory (Table 1). Carcasses of 11 dogs (2 poodles, 2 Staffordshire terriers, 1 Pekinese, 1 bull mastiff, 1 Walker hound, 2 Doberman pinschers, 2 mixed breed dogs) that died with clinical signs suggestive of canine parvoviral enteritis were presented for necropsy. Tissues, including tongue, were fixed in neutral buffered formalin and embedded in paraffin. Blocks were sectioned at 5 μ m, stained with HE, and examined microscopically. Unfixed intestinal contents and tongue specimens were submitted for

negative stain examination for viruses by electron microscopy. For ultrastructural examination, formalin-fixed tongue samples from all but dog 1 were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) and embedded in epoxy. Tissue for dog 6 was retrieved from the paraffin block. Ultrathin sections were stained with uranyl acetate and lead citrate. Additional sections of unfixed tongue and small intestine were examined using direct immunofluorescence with fluorescein isothiocyanate-conjugated anti-CPV-2 antibody.^a Tongue epithelium from 4 dogs was also submitted for virus isolation.

Gross and histologic lesions associated with CPV-2 enteritis have been well described.^{1,2,6,7} Intestinal lesions compatible with parvoviral enteritis were found in all 11 dogs. There was necrosis of crypt epithelium, villous atrophy, stromal collapse, and hemorrhage. Rare basophilic intranuclear inclusion bodies were found in the small intestine of some dogs. Large, lightly basophilic to amphophilic inclusion bodies, frequently surrounded by a clear halo, were observed in numerous glossal epithelial cells in all 11 dogs. In contrast to the previous report of glossal CPV-2 inclusion bodies,⁴ these inclusions were located by light microscopy in the cytoplasm (Fig. 1). Only rare basophilic intranuclear inclusions were observed in glossal epithelium. The cytoplasmic inclusion bodies often displaced the nucleus to the cell periphery.

Examination of homogenized tongue by negative-stain electron microscopy demonstrated parvovirus in 4 of 7 dogs (Fig. 2). Ultrastructural examination of sections 60–90 nm thick revealed numerous inclusions consistent with those observed by light microscopy (Fig. 3A). Most inclusions were composed of closely packed, hexagonal, electron-dense particles, approximately 20 nm, intermixed with rare empty capsids. The appearance of the particles was consistent with parvovirus (Fig. 3B). Some inclusions in dogs 5, 8, 9, and 11 contained widely scattered virions occasionally intermixed with scant granular/fibrillar matrix (Fig. 3B) or, rarely

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