Journal of Zoo and Wildlife Medicine 21(1): 84–87, 1990

ACUTE NECROTIZING ENTERITIS ASSOCIATED WITH SUSPECTED CORONAVIRUS INFECTION IN THREE HARBOR SEALS (*PHOCA VITULINA*)

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Abstract: Two adult harbor seals (*Phoca vitulina*) died without signs of disease, and a third seal exhibited marked leukocytosis, dehydration, hypernatremia, and hyperchloremia. Significant pathologic findings were indicative of acute necrotizing enteritis and pulmonary edema. Fluorescent antibody staining of small intestinal tissue from two of the affected seals was positive with antisera to porcine transmissible gastroenteritis virus, feline infectious peritonitis virus, and canine enteric coronavirus, but negative for bovine coronavirus and canine parvovirus. The gross, microscopic, and immunofluorescent findings in this case were similar to enteritis associated with coronavirus infections in other mammalian species.

Key words: Enteritis, coronavirus, harbor seal, *Phoca vitulina.*

CASE REPORT

In August 1987, an adult female and male harbor seal (*Phoca vitulina*) died suddenly without signs of disease. The seals were housed with another adult male harbor seal and three adult California sea lions (*Zalophus californianus*) in a group that had been established for 3 yr. These pinnipeds were kept in an outdoor exhibit that consisted of an 18,494-L (70,000-gal) natural ocean-water pool with an approximately 200-m^2 concrete dry resting area. Pool water was pressure filtered through a sand–gravel mixture, and chlorine was maintained between 0.4 and 0.9 ppm. Fecal coliforms were routinely <100 most probable number/100 ml of water.

The seals were each fed 2.5–3.5 kg of table quality smelt, herring, and mackeral daily and supplemented with a multivitamin preparation (Stress Formula 600, Goldline Laboratories, Ft. Lauderdale, Florida 33309, USA) and 100 mg of thiamine. The 15-yr captive health history of all seals was unremarkable.

Two days after the first two seals died, the remaining male harbor seal was examined because of anorexia and abnormal behavior. Moderate, moist, diffuse rales were auscultated in both lung fields. Mucous membranes were purplish red and injected.

Blood was taken from an extradural intravertebral vein for hematologic and serum chemical evaluation. Blood and fresh feces were cultured for aerobic bacteria.

An intravenous catheter was placed in an interdigital venous plexus and 1 L of 2.5% dextrose in half-strength lactated Ringer's solution with 500 mg amikacin (Amiglyde-V, Aveco Co., Fort Dodge, Iowa 50501, USA) was administered in the first hour followed by 0.75 L of fluids alone in the second hour; 600,000 IU procaine penicillin G (Burns Veterinary Supply, Oakland, California 94621, USA) was administered i.m. The seal died approximately 5 hr after therapy was begun.

The seal had a marked leukocytosis (42,500 WBC/μl; normal range for this seal was 7,000–9,000 WBC/μl) with absolute neutrophilia (39,525 neutrophils/μl; normal range for this seal was 2,660–6,480 neutrophils/μl). The hematocrit was 73% (normal range for this seal was 50–60%). The remaining leukogram and erythrogram were

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within normal established ranges for this seal.

The seal was hypernatremic (177 mEq/L; normal range for this seal was 147–156 mEq/L) and hyperchloremic (>130 mEq/L; normal range for this seal was 100–110 mEq/L). The serum urea nitrogen was 189 mg/dl (normal range for this seal was 44–60 mg/dl) and the lactic dehydrogenase was >1,500 mg/dl (normal range for this seal was 240–483 mg/dl). The serum ALT, AST, total bilirubin, creatinine, cholesterol, alkaline phosphatase, phosphorus, calcium, total protein, albumin, globulin, potassium, CO_2, gamma glutamyl transpeptidase, uric acid, amylase, and lipase were within established ranges for this seal. No bacteria were isolated from the blood cultures and *Pseudomonas putrefaciens* was isolated in pure culture from the feces. The organism was sensitive to many antibiotics, including amikacin, gentamicin, tetracycline, and penicillin.

Pathologic findings

Necropsies performed on the three seals, each within 1 hr of death, all showed markedly heavy, wet, and diffusely magenta to purple lungs, which exuded moderate frothy pink serous fluid from the cut surfaces. The small intestines of each seal also had moderate serosal petechiation, and the mucosal linings appeared diffusely atrophic, deep red, and were easily removed with light digital pressure. In addition, the female seal had a white firm multicystic mass approximately 12 × 7 × 7 cm in size occupying a part of the left ovary that was contiguous with the left uterine horn.

Cultures for aerobic bacteria were obtained from heart blood, lungs, and the small intestines of two seals. All blood cultures were negative. The intestinal cultures yielded mixed growths of *P. putrefaciens, Escherichia coli,* and *Enterobacter cloacae,* which were interpreted as normal intestinal flora; one lung culture had a moderate growth of *E. cloacae.* Segments (approximately 2 cm in diameter) of small intestine from the female and the longest surviving male seal were frozen at −70°C for virologic studies. Sections of representative organs from the three seals were fixed in neutral 10% buffered formalin and processed routinely for histologic evaluation.

Histopathologic examination of the small intestines of the three affected seals revealed moderate to severe enterocyte necrosis with villous atrophy. Some villi were fused and had denuded tips (Fig. 1). The mucosal brush border was irregularly absent. The lamina propria had extensive infiltrates of polymorphonuclear leukocytes and increased numbers of lymphocytes and plasma cells. There was also mucosal and subserosal hemorrhage. No microorganisms considered to be causative were observed in any of the lesions. The lungs of all seals showed extensive focal bronchoalveolar hemorrhage and edema, and severe diffuse pulmonary congestion. The spleens and visceral and peripheral lymph nodes from all three of the affected seals had moderate to severe lymphoid depletion. The nodal depletion involved follicular and paracortical lymphoid tissue. The mass in the left ovary of the female seal was determined to be an ovarian papillary cystadenocarcinoma without microscopic evidence of metastases.

Virological studies

The frozen mucosa of the small intestinal tissue from the two affected seals was thawed, scraped, smeared on microscope slides, blotted, and air dried then fixed in acetone for 15 min. For direct fluorescent antibody staining, fluorescene-conjugated antiserum at a 1:40 dilution against transmissible gastroenteritis virus and bovine coronavirus (obtained from National Veterinary Service Laboratories, Ames, Iowa 50010, USA) and feline infectious peritonitis virus and canine parvovirus (obtained from Specialized Assays, Inc., Milton, Pennsylvania 17847, USA) was flooded on the fixed slides and incubated for 30 min in

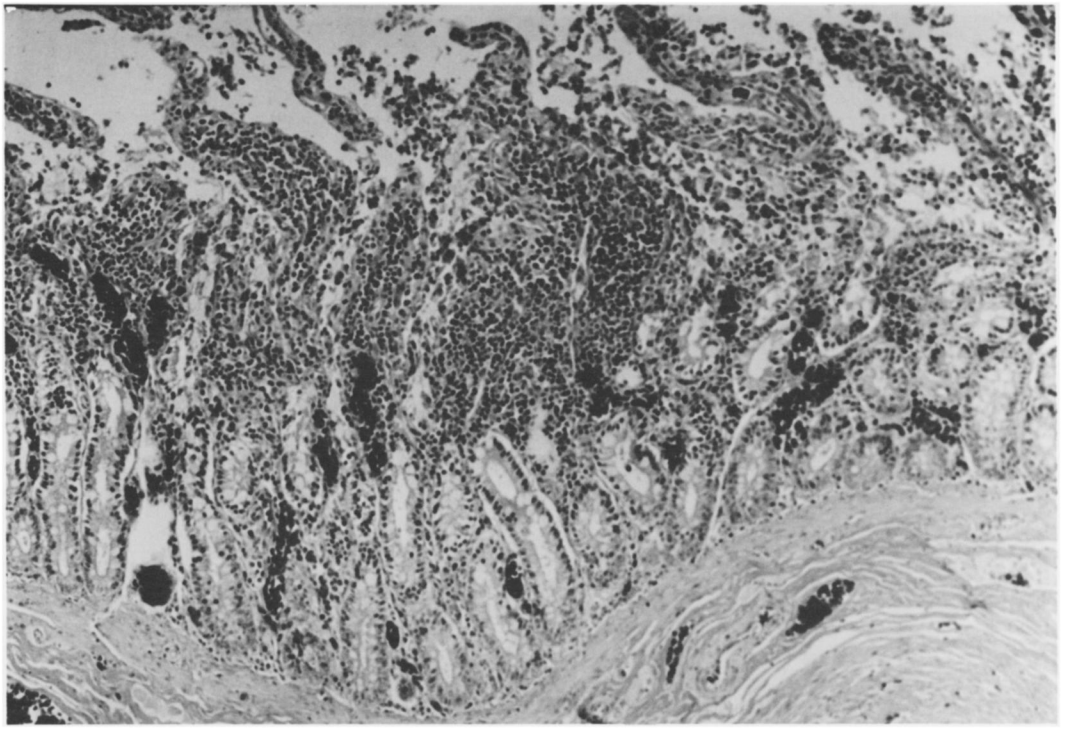

Figure 1. Photomicrograph of the small intestine from a female harbor seal with suspected coronavirus enteritis. Note the necrosis of the upper segments of the villi with fusion of the lamina propria, which is heavily infiltrated with inflammatory cells. There is also focal mucosal congestion and hemorrhage. H&E, ×100.

a moist chamber. Slides were then rinsed in phosphate-buffered saline, and 1% Evans blue at a dilution of 1:500 was added as a counter stain.

For indirect fluorescent antibody staining, the fixed slides were flooded with a 1:40 dilution of serum from a dog that was positive for canine coronavirus with a titer of 40 as determined by indirect immunofluorescence. The seal slides were incubated for 30 min, rinsed with phosphate-buffered saline for 15 min, flooded with conjugated anti-canine IgG antiserum (obtained from Cappel, Malvern, Pennsylvania 19355, USA), incubated for 30 min, and rinsed and washed as above. Slides were then viewed under a fluorescent microscope for fluorescence.

Fluorescent antibody staining for transmissible gastroenteritis virus, feline infectious peritonitis virus, and canine coronavirus was positive. Fluorescent antibody staining for bovine coronavirus and canine parvovirus was negative.

In addition, samples of the frozen intestinal tissue from two affected seals were ground-up and filtered at 0.45 μm. After the addition of 0.5 mg/100 ml of gentamicin, the ground tissue filtrate was inoculated onto standard tissue cultures of bovine kidney, monkey kidney, Crandell feline kidney, Martin Darby canine kidney, and rabbit kidney. No viral cytopathic effects (CPE) were observed after 2 wk and one passage.

DISCUSSION

The histopathologic changes and positive immunofluorescence with antisera to feline infectious peritonitis virus, transmissible gastroenteritis virus, and canine coronavirus were considered supportive of an etiologic diagnosis of coronavirus enteritis. The histologic lesions of coronavirus enteritis in swine, cattle, dogs, and cats include villous

atrophy and fusion.[2–4] The mucosal brush border is irregularly lost and inflammatory cell infiltrates of the lamina propria are generally not severe. The enteric lesions in the seal cases were similar with the exception of an extensive and diffuse admixture of inflammatory cell infiltrates in the lamina propria. In addition, the histologic pattern of the lymphoid depletion present in the three seals was suggestive of compromise to both humoral and cell-mediated immune systems.

The absence of CPE in the tissue cultures utilized does not rule out coronavirus as the possible causative agent. These findings were not surprising because most coronaviruses for isolation are rather host-specific.[6]

The positive immunofluorescent results for three different species of coronavirus are also not considered unusual as coronaviruses frequently cross-react.[1,4] Because false-positive immunofluorescent results can occur as a result of non-specific antibody binding, only a presumptive diagnosis can be made in this case.[1,5] A definitive diagnosis of coronavirus enteritis would require viral isolation preferably in harbor seal tissue cultures, which are not readily available.

The absence of diarrhea in the harbor seal cases, which is a typical sign in other species with coronavirus enteritis, may reflect the peracute nature of this infection in the seals. The source of infection and method of transmission of an apparently virulent agent in an isolated established group of pinnipeds remain speculative. A feline source cannot be ruled out because feral cats were found at this oceanarium prior to and during the disease outbreak. The absence of clinical disease in the sea lions occupying the same exhibit suggests that this disease may be species-specific among pinnipeds.

Acknowledgment: We thank the animal care staff and management of Ocean World, Ft. Lauderdale, Florida, for their assistance in this case.

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Received for publication 30 August 1988.