Vol. 120: 189–194, 2016
doi: 10.3354/dao03019

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 9

Acute necrotizing colitis with pneumatosis intestinalis in an Amazonian manatee calf

Guilherme Guerra Neto[1,*], Marina Galvão Bueno[1,2], Rodrigo Otavio Silveira Silva[3], Francisco Carlos Faria Lobato[3], Juliana Plácido Guimarães[4], Gregory D. Bossart[5,6], Miriam Marmontel[1]

[1]Instituto de Desenvolvimento Sustentável Mamirauá, Estrada do Bexiga 2584, 69553-225 Tefé, AM, Brazil
[2]Fundação Oswaldo Cruz – Fiocruz, Av. Brasil 4036, sala 214 - Prédio da Expansão, 21040-361 Rio de Janeiro, RJ, Brazil
[3]Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Medicina Veterinária Preventiva, Av. Antônio Carlos 6627, Caixa Postal 567, campus Pampulha da UFMG, 31270-901 Belo Horizonte, MG, Brazil
[4]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Cirurgia, 05508-270 São Paulo, SP, Brazil
[5]Animal Health, Research and Conservation, Georgia Aquarium, 225 Baker St. NW, Atlanta, GA 30313, USA
[6]Division of Comparative Pathology, Miller School of Medicine, University of Miami, PO Box 016960 (R-46), Miami, FL, USA

ABSTRACT: On 25 January 2014, a 1 mo old female Amazonian manatee *Trichechus inunguis* calf weighing 12 kg was rescued by air transport in Guajará, Brazil, and transferred to Mamirauá Institute's Community-based Amazonian Manatee Rehabilitation Center. The calf presented piercing/cutting lesions on the back, neck, and head, in addition to dehydration and intermittent involuntary buoyancy. X-ray analysis revealed a large amount of gases in the gastrointestinal tract. Daily procedures included wound cleaning and dressing, clinical and laboratory monitoring, treatment for intestinal tympanism, and artificial feeding. Adaptation to the nursing formula included 2 kinds of whole milk. Up to 20 d post-rescue the calf presented appetite, was active, and gained weight progressively. Past this period the calf started losing weight and presented constant involuntary buoyancy and died after 41 d in rehabilitation. The major findings at necropsy were pneumatosis intestinalis in cecum and colon, pulmonary edema, and hepatomegaly. The microscopic examination revealed pyogranulomatous and necrohemohrragic colitis with multinucleated giant cells, acute multifocal lymphadenitis with lymphoid depletion in cortical and paramedullary regions of mesenteric lymph nodes, and diffuse severe acinar atrophy of the pancreas. Anaerobic cultures of fragments of cecum and colon revealed colonies genotyped as *Clostridium perfringens* type A. We speculate that compromised immunity, thermoregulatory failure, and intolerance to artificial diet may have been contributing factors to the infection, leading to enterotoxemia and death.

KEY WORDS: *Trichechus inunguis* · Rehabilitation · Health · Conservation · *Clostridium perfringens*

INTRODUCTION

Gastrointestinal disorders are the most frequent clinical findings to occur at the onset of the rehabilitation period of orphaned Amazonian manatee *Trichechus inunguis* and West Indian manatee *T. manatus* calves in Brazil, Florida (USA), and Puerto Rico (Bossart 2001, Lazzarini et al. 2014). Enterocolitis with pneumatosis intestinalis is considered to be the most severe among these, and if undiagnosed, may rapidly evolve to sepsis, shock, and death (Walsh et al. 1999). Factors associated with the post-rescue initial stress to captivity, such as low immunity, thermoregulatory failure, artificial formulae, and acquired

*Corresponding author: guilhermeguerraneto@hotmail.com

infections, are suspected to be the main causes of this condition in Florida manatees (Bossart 2001, Bossart et al. 2002). In humans, necrotizing enterocolitis is among the most severe gastrointestinal emergencies during the neonatal period; it is a disease of unknown etiology, which affects mainly premature newborns, and may be associated with pneumatosis intestinalis as a sequel (Thompson & Bizarro 2008). The objective of this report was to characterize acute enterocolitis with concomitant pneumatosis intestinalis in an Amazonian manatee calf.

CASE REPORT

On 25 January 2014 (Day 1), an approximately 1 mo old (total straight length = 85 cm; weight = 12 kg) female Amazonian manatee calf was rescued by aerial transport in the town of Guajará (7° 11′ 31″ S, 72° 48′ 15″ W), in the Western Brazilian Amazon, under permit 05/2014/GTFauna/IBAMA/AM. The person then keeping the calf reported having maintained the animal for 15 d on 5 daily bottles of Nan® 2 (Nestlé; concentration 6.5 g in 100 ml of water) and common water hyacinth *Eichhornia crassipes* offered ad libitum. A physical examination revealed several punctiform and penetrating cutting lesions approximately 0.5 cm wide and 0.3 cm deep on the back, neck, and head areas; in addition, the animal was emaciated and dehydrated. During the 3 d immediately post-rescue, the calf was maintained in a quarantine pool in the town of Tefé, Brazil (3° 21′ 25″ S, 64° 43′ 06″ W).

Radiographic examination showed a large amount of air in the gastrointestinal tract. An artificial milk formula was implemented on the first day of rescue and consisted of 15 g of non-lactose Nan® (Nestlé) and 10 g of Nan® 2 diluted in 150 ml of mineral water at a concentration of 16.66 g per 100 ml. The formula was offered by bottle 6 times a day, in addition to the aquatic macrophytes *Paspalum repens* and *E. crassipes* ad libitum. The calf showed suckling reflex and was nursing voluntarily. Stomach tubing was performed twice, consisting of 60 ml sodium chloride solution at 0.9% and 60 ml glucose solution at 5% on the first and second days of rehabilitation. The calf presented intermittent involuntary buoyancy of the lumbar region on the second day of rehabilitation, and therefore simethicone (Flaticon®, Belfar; 1.77 mg kg^{-1} per os) was added to each bottle. Cleaning and dressing of penetrating skin lesions was conducted twice a day with chlorhexidine solution (Riohex® 2%, Rioquímica) and healing and antiseptic cream (Alantol®, Furanil®, and Enterex®, Vetnil; Corega®, GSK), specifically prepared for use in water. On Day 4, the calf weighed 12 kg.

On Day 7, the calf was transferred to the Community-based Amazonian Manatee Rehabilitation Center of the Mamirauá Institute of Sustainable Development (MISD) (2° 29′ 27″ S, 64° 43′ 03″ W), where it underwent another clinical examination, blood sampling for complete blood counts (CBC), and wound dressing. White blood cell (WBC) counts did not reveal a leukocytosis (WBC: 11.200 mm^{-3}), but CBC results suggested dehydration (62% hematocrit), which was treated with oral fluid therapy via orogastric probe. Another gradual change was made to the milk formula, replacing Nan® 2 with whole cow milk (Ninho®, Nestlé), and the calf was offered 6 bottles daily of 120 ml formula (concentration 20 g per 100 ml water); 2 g of probiotics (Pro-biótico Cães e Gatos®, Vetnil) and 2 ml of multivitamin (Glicopan®, Vetnil) were added to the first bottle of the day, and 12 drops of simethicone (1.77 mg kg^{-1}) to each bottle.

Up to mid-February the calf showed appetite, gained 0.8 kg, was active, and showed no signs of involuntary buoyancy. Skin lesion healing was progressive, and feces had firm consistency and normal color. Nevertheless, after 20 d in rehabilitation, the calf exhibited constant buoyancy and increased respiratory rate to 1–2 breaths min^{-1}. Milk formula, in the same concentration, was offered in reduced volume, as there was 20 to 50 ml waste at each bottle offered. Bloat treatment was maintained. Feces maintained normal aspect in color and consistency; however, the calf lost 1.4 kg body weight. On Day 36, CBC indicated dehydration and light leukocytosis. From Day 40 onwards, although still nursing, the calf appeared listless with no reaction to handling. The calf died in the morning of Day 41. Necropsy was conducted immediately, following criteria recommended by Bonde et al. (1983).

RESULTS

Macroscopic findings were emaciation, presence of petechiae in the subcutaneous tissue of the thorax and abdomen between the skin and panniculus muscles, and intense congestion of the vessels in the serosa. Moreover, we observed a large amount of air bubbles inside the wall of the cecum (Fig. 1A), colon (Fig. 1B), and mesentery (Fig. 1C), areas of lung congestion alternating with areas with air bubbles and hepatomegaly. Notable microscopic findings were

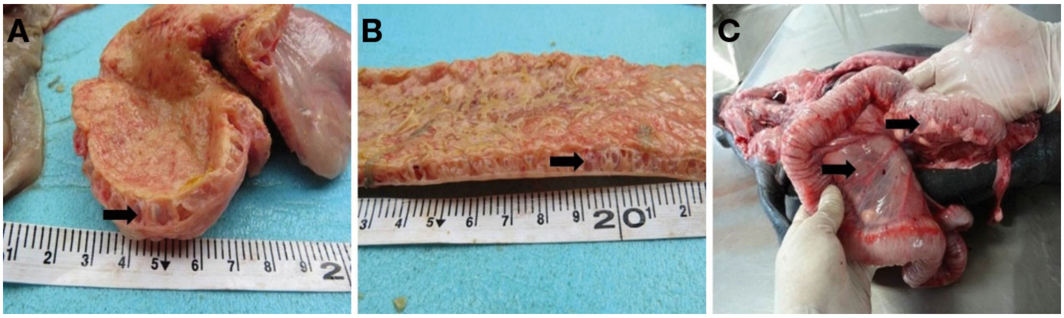

Fig. 1. Pneumatosis in the (A) cecum and (B) colon (arrows) of a 1 mo old female Amazonian manatee *Trichechus inunguis* calf. (C) Mesentery presenting congested vessels and air bubbles (arrows). Photos by G. Guerra Neto

present in the cecum and colon. Large variably-sized gas bubbles were present in the submucosa (Fig. 2A) surrounded by an admixture of macrophages, lymphocytes, neutrophils, and occasional multinucleated cells (Fig. 2B,C). Multifocal mucosal necrosis and hemorrhage were also present. Occasional intralesional coccobacilli were present. A mesenteric lymph node had moderate to severe multifocal sinusoidal neutrophilia with lymphoid depletion in cortical and paramedullary regions. The remaining microscopic lesions included severe diffuse acinar atrophy of the pancreas, severe renal congestion and moderate multifocal pulmonary emphysema and congestion.

Samples of fragments and contents of cecum and large intestine were frozen at 20°C and then processed for aerobic and anaerobic bacterial testing, according to standard techniques (Silva et al. 2013). Briefly, the samples were submitted for *Salmonella* spp. culture in Hektoen Enteric Agar (Prodimol Biotechnology); isolation of *Escherichia coli* in MacConkey agar (Prodimol Biotechnology) followed by a previously described PCR method (Puño-Sarmiento et al. 2013) to investigate the following virulence factor genes: *eae*, *stx1*, *stx2*, *ehxA*, *aggR*, *ipaH*, *eltA*, *etlB*, *est*, and *stap*. *Salmonella* spp. were not isolated, and *E. coli* isolates were negative for all virulence factors investigated. SPS agar (Difco) was used to investigate the presence of *Clostridium perfringens*. After anaerobic incubation for 24 h, 5 colonies with suggestive morphology were individually subjected to previously described PCR protocols for genotyping and investigation of additional virulence factors (Keyburn et al. 2008, Silva et al. 2014, Gohari et al. 2015). The isolated strains were genotyped as *C. perfringens* type A (positive for alpha toxin-encoding gene) and negative for the following genes: *cpb2*, *cpe*, *netB*, *netE*, and *tpeL*.

DISCUSSION

This is the first case report of necrotizing enterocolitis with pneumatosis intestinalis in *Trichechus inunguis*. Its importance is directly related to the large number of calves sent for rehabilitation with different

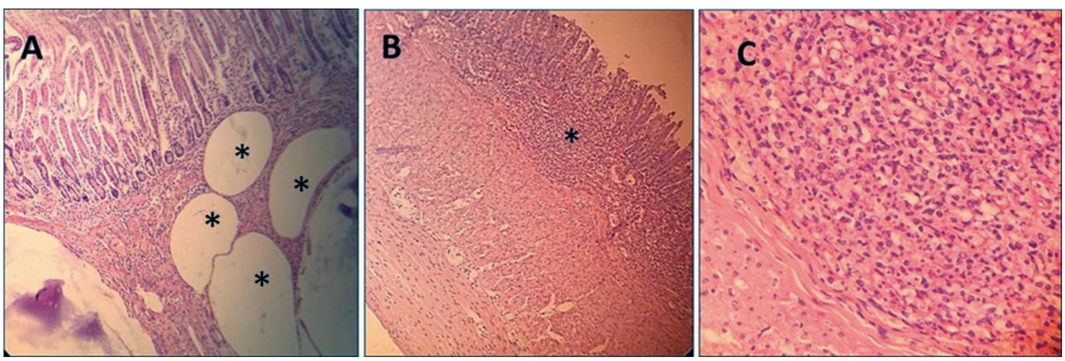

Fig. 2. Microscopic findings in the cecum and colon of a 1 mo old female Amazonian manatee *Trichechus inunguis* calf. (A) Pneumatosis intestinalis in the colon, showing variably-sized submucosal gas bubbles (asterisks); hematoxylin and eosin (H&E) stain (10×). (B) Mixed inflammatory cell infiltrate (asterisk) of submucosa in the colon; H&E (10×). (C) Polymorphonuclear inflammatory cell infiltrate in the cecum; H&E (40×). Photos by M. Galvão Bueno

clinical problems which, if not already established, may still develop during the rehabilitation period.

According to Walsh et al. (1999), orphan manatees in rescue facilities can present a number of problems including malnutrition, dehydration, hypogammaglobulinemia, sepsis, trauma, and enterocolitis. Among all common intestinal complications, necrotic enterocolitis and pneumatosis intestinalis are the most severe. Pneumatosis intestinalis is an intramural accumulation of gas in the intestinal wall that can occur from the stomach to the colon, ranging from focal to linear involvement. Etiologies in humans may include bowel necrosis due to ischemia, infarction, necrotizing enterocolitis, neutropenic colitis, volvulus, and sepsis. Pneumatosis can be secondary to mucosal disruption caused by ulceration, mucosal erosion, trauma, perforation, and defects in the intestinal crypts secondary to acute and subclinical enteritis that allows intraluminal bacterial gas to percolate into the bowel wall. Treatment options may include surgical removal of the affected area or complete rest of the intestinal tract with parenteral hyperalimentation. The limitations of these techniques include malabsorption secondary to extensive bowel loss, and difficulties with maintenance of sterile intravenous line for extended periods of time in a mobile animal. Necropsy results of previous cases show a wide variation in the amount of the colon involved, although pneumatosis intestinalis can extend from the rectum up to, and including, the cecum.

According to Lazzarini et al. (2014), gastrointestinal diseases are the most frequent illnesses in captive manatees in Brazil, frequently with non-diagnostic clinical signs; when they become evident, the situation may be irreversible. As in the present case, gastrointestinal problems are common during the adaptation period of a rescued manatee calf to the artificial nursing formula. Nevertheless, the calf was being monitored on a daily basis, and up to 20 d post-rescue, it demonstrated appetite and weight gain. It was past this period that the calf showed lack of appetite and increased involuntary buoyancy, despite non-diarrheic feces. It is likely that the increase in respiratory frequency observed in the calf's last days of life is associated with the rapid increase in the amount of intestinal gas that compressed the lungs. Although the acute enterocolitis was not diagnosed ante mortem, prophylactic administration of antibiotics may have prevented the observed complications.

Cases of enterocolitis in cachectic and hypothermic West Indian manatee calves with metabolic exhaustion, and dying of shock or sepsis, have been reported in rehabilitation centers in Florida (Walsh & Bossart 1999, Bossart 2001). Bossart (2001) speculated that the etiology of this clinical condition is multiactorial, including the immunological condition, the presence of infectious disease agents, and artificial nursing formulae.

Studies have shown that approximately 90% of human neonates that develop enterocolitis have received artificial milk formula (Kliegman 1998). The main causal factors of the illness are hypothesized to be related to hyper-osmolarity, lack of immunoprotective factors in artificial formulae, the volume and speed of administration of formula, and an abnormal immune response to proteins in the artificial milk (Willis et al. 1977).

Several studies on different nursing formulae given to manatee calves have been conducted with the goal of attaining a good organic recovery to the rehabilitation process and, later, a healthy development of calves (Askin et al. 2014). Enteral diets for newborn humans were used in the only 2 cases of pneumatosis intestinalis associated with necrotizing enterocolitis treated with success in Florida manatees. The first case was reported by Walsh et al. (1999) and the treatment was based on the use of enteral diet with Criticare HN® and Nutramigen® (Mead Johnson and Co.), in addition to supportive therapy. Three weeks after the end of therapy, the calf resumed the regular nursing formula but presented hemorrhagic colitis, which was treated for 14 d with antibiotics, probiotics containing *Lactobacillus*, fresh feces from adult manatees, and antifungal substances. In the second case, Tollefson & Croft (2011) also treated a Florida manatee calf afflicted with pneumatosis intestinalis with enteral diet consisting of 100% Nutramigen®; although the calf presented intestinal improvement, it did not gain weight. Diet was then transferred to Elecare® (Abbott). Two months later, when a combination of oils (palm, macadamia, and coconut) in the proportion of 2:2:1 was added, in association with medium-chain fatty acids, the calf started gaining weight progressively.

The basis of the artificial formula offered to calves in MISD's Rehabilitation Center has always been powdered whole cow milk diluted in water. Although transitory diarrhea could occur in the first month of rehabilitation, severe intestinal illnesses never occurred. Nevertheless, as each case is unique, the osmolarity of the powdered whole milk offered to the calf in this study from the fifth day on may have contributed to the development of enterocolitis with pneumatosis. As lactose is naturally lacking or present only in small amounts in *T. inunguis* milk (Barbosa 2011), the elevated osmolarity and/or overnutrition could result in malabsorption of lactose carbohydrates; this would increase the concentration of this sugar in the large intestine, metabolized by bacteria, with production of hydrogen and short-chain organic acids extremely harmful to the intestinal mucosa, leading to the development of enterocolitis (McKeown et al. 1992). With the disruption of the mucosal barrier, bacteria penetrated the intestinal wall, and gas production by the bacteria caused the formation of pneumatosis. The nursing formula used in this study had 3% carbohydrates, whereas Bossart (2001) stated that manatee calves can tolerate diets with a maximum of 5% carbohydrates.

Another factor that needs attention is the temperature of the water in the quarantine pool in which the animal was held. In the Central Amazon, the first trimester of the year is characterized by a high pluviometric index, and average temperature during this period is between 25 and 26°C (Instituto Nacional de Meteorologia, www.inmet.gov.br). However, the minimum temperatures dropped to 21°C in January and to 19–20°C in February and March. Whereas the ideal temperature in manatee calf rehabilitation pools should be between 27 and 30°C (Lazzarini et al. 2014), the low temperature at night likely led to a hypothermic condition in this animal. Sirenians have a low metabolic rate and a limited capacity of heat production, and they do not tolerate cold water for long periods of time (Gallivan et al. 1983, Irvine 1983). Although sirenians have high immunological competence, exposure to cold water can lead to development of hypothermia and cold stress syndrome, compromising the immunological system and predisposing it to infections (Bossart et al. 2002). The observed lymphoid depletion affects both humoral and cell-mediated immunological systems, as it occurs both in cortical and medullary areas of lymph nodes (Bossart et al. 2002). This was observed in the histopathological analysis of mesenteric lymph nodes of the calf in this case. Reduced lymphocyte production may contribute to an excessive and prolonged release of glucocorticoids related to stress and nutritional deficiencies, suppressing the immunological system of the patient and making it susceptible to infections (Halvorsen & Keith 2008).

Other pathological findings in this study include atrophy of pancreatic acini and enterocolitis. The acinar atrophy of the pancreas suggests prolonged inanition due to protein-caloric deficiency and malabsorption (Jubb 1993); this was a sequel to the hindgut lesions, although this may also be an artifact of a diet that was not working. It is therefore possible that the calf in this case may have been victim to hypothermia which contributed to the exacerbation of a preexisting infection.

Clostridium perfringens was isolated from the affected animal. This microorganism is commonly found as part of the gastrointestinal microbiota of humans and animals, including healthy wild harbor seals *Phoca vitulina*, but is also associated with intestinal disease in several domestic and wild species (Greig et al. 2014, Silva & Lobato 2015). *C. perfringens* is classified into 5 toxigenic types, from A to E, depending on the production of 4 major toxins: alpha, beta, epsilon, and iota (Songer 2010). All strains isolated in the present study were genotyped as type A (positive for alpha toxin-encoding gene), but negative for all additional virulence factors tested, including enterotoxin, which is associated with gastrointestinal disease in humans, and NetB and NetE, 2 pore-forming toxins associated with necrotic enteritis in broiler chickens, dogs, and horses (Keyburn et al. 2008, Gohari et al. 2015, Silva & Lobato 2015). It is important to note that for most wild species, the role of *C. perfringens* as a pathogen or even as part of the normal microbiota is still poorly understood (Silva & Lobato 2015). This report highlights the need for more studies in order to clarify the role of *C. perfringens* in manatee calves, which could help us prevent gastrointestinal disorders in these animals.

Interestingly, pathological findings in this case are similar to those often seen in orphaned Florida manatees under rehabilitation (Bossart et al. 2004). Following the example of Florida, the use of artificial nursing formulae established for premature human newborns must be considered in Brazil, in addition to the use of thermostats to control the temperature in the quarantine pools. We hypothesize that the compromised immunity, the limited thermoregulatory capacity associated with nighttime low temperatures, and the intolerance to the artificial nursing formula were the contributing factors to the infection by *C. perfringens*, leading to enterotoxemia and death. This work contributes to the knowledge on management and maintenance of Amazonian manatee calves in rehabilitation, and the conservation of the species.

Acknowledgements. We thank the National Council for Scientific and Technological Development (Conselho Nacional de Pesquisa e Inovação–CNPq) and the Ministry of Science, Technology and Innovation (Ministério da Ciência, Tecnologia e Inovação–MCTI) for their financial support, and the Mamirauá Sustainable Development Institute (Instituto de

Desenvolvimento Sustentável Mamirauá – IDSM) for logistical support. We also thank all members of the Research Group on Amazonian Aquatic Mammals.

LITERATURE CITED

Askin N, Belanger M, Wittnich C (2014) A review of natural milk, commercial replacement formulae, and homemade substitutes used in the care of rescued manatee calves. J Mar Anim Ecol 7:17–22

Barbosa PS (2011) Composição química do leite de peixe-boi da Amazônia (*Trichechus inunguis* Natterer 1883) em cativeiro nos diferentes estágios de lactação. MSc thesis, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus

Bonde R, O'Shea T, Beck C (1983) A manual of procedures for the salvage and necropsy of carcasses of the West Indian manatee (*Trichechus manatus*). Document No. PB83–255273. National Technical Information Service, Springfield, VA

Bossart GD (2001) Manatees. In: Dierauf L, Gulland F (eds) Marine mammal medicine. CRC Press, Boca Raton, FL, p 939–960

Bossart GD, Meisner R, Rommel SA, Ghim S, Jenson AB (2002) Pathological features of the Florida manatee cold stress syndrome. Aquat Mamm 29:9–17

Bossart GD, Meisner R, Rommel SA, Lightsey JA, Varela RA, Defran RH (2004) Pathologic findings in Florida manatees (*Trichechus manatus latirostris*). Aquat Mamm 30: 434–440

Gallivan G, Best R, Kanwisher J (1983) Temperature regulation in the Amazonian manatee, *Trichechus inunguis.* Physiol Zool 56:255–262

Gohari IM, Parreira VR, Nowell VJ, Nicholson VM, Oliphant K, Prescott JF (2015) A novel pore-forming toxin in type A *Clostridium perfringens* is associated with both fatal canine hemorrhagic gastroenteritis and fatal foal necrotizing enterocolitis. PLOS ONE 10:e0122684

Grieg JD, Gulland FMD, Smith WA, Conrad PA and others (2014) Surveillance for zoonotic and selected pathogens in harbor seals *Phoca vitulina* from central California. Dis Aquat Org 111:93–106

Halvorsen KM, Keith EO (2008) Immunosuppression cascade in the Florida manatee (*Trichechus manatus latirostris*). Aquat Mamm 34:412–419

Irvine AB (1983) Manatee metabolism and its influence on distribution in Florida. Biol Conserv 25:315–334

Jubb K (1993) The pancreas. In: Jubb K, Kennedy P, Palmer N (eds) Pathology of domestic animals, Vol 2. Academic Press, New York, NY, p 410–411

Keyburn AL, Boyce JD, Vaz P, Bannam TL and others (2008) NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens.* PLoS Pathog 4:e26

Kliegman RM (1998) Pathophysiology and epidemiology of necrotizing enterocolitis. In: Polin RA, Fox WW (eds) Fetal and neonatal physiology, 2nd edn. Saunders Company, Philadelphia, PA, p 1425–1432

Lazzarini SM, Vergara-Parente JE, Ribeiro DC (2014) Sirenia (Peixe-boi-da-Amazônia e Peixe-boi-marinho). In: Cubas ZS, Silva JCR, Catão-Dias JL (eds) Tratado de animais selvagens: medicina veterinária. Roca, São Paulo, p 936–972

McKeown RE, Marsh D, Amarnath V (1992) The role of delayed feeding and feeding increment in necrotizing enterocolitis. J Pediatr 121:764–770

Puño-Sarmiento J, Medeiros L, Chiconi C, Martins F and others (2013) Detection of diarrheagenic *Escherichia coli* strains isolated from dogs and cats in Brazil. Vet Microbiol 166:676–680

Silva ROS, Lobato FCF (2015) *Clostridium perfringens*: a review of enteric diseases in dogs, cats and wild animals. Anaerobe 33:14–17

Silva ROS, Lobato FCF, Pires PS, Gabardo MP, Paladino ES, Guedes RMC (2013) Neonatal diarrhea in a piglet associated with cpb-2 positive *Clostridium perfringens.* Braz J Vet Pathol 6:11–14

Silva ROS, D'Elia ML, Teixeira EPT, Pereira PLL and others (2014) *Clostridium difficile* and *Clostridium perfringens* from wild carnivore species in Brazil. Anaerobe 28: 207–211

Songer JG (2010) Clostridia as agents of zoonotic disease. Vet Microbiol 140:399–404

Thompson AM, Bizarro MJ (2008) Necrotizing enterocolitis in newborns: pathogenesis, prevention and treatment. Drugs 68:1227–1238

Tollefson TN, Croft LA (2011) Hand raising manatee (*Trichechus manatus*). In: Ward A, Coslik A, Maslanka M (eds) Proc 9th Conf Zoo Wildl Nutr. AZA, Nutrition Advisory Group, Kansas City, MO

Walsh MT, Bossart GD (1999) Manatee medicine. In: Fowler ME, Miller RE (eds) Zoo and wildlife medicine. W.B. Saunders, Philadelphia, PA, p 507–516

Walsh MT, Murphy D, Innis SM (1999) Pneumatosis intestinalis in orphan manatees (*Trichechus manatus latirostris*): diagnosis, pathological findings and potential therapy. In: Proc 30th Conf Annu Int Assoc Aquat Anim Med, p 1

Willis DM, Chabot J, Radde IC (1977) Unsuspected hyperosmolality of oral solutions contributing to necrotizing enterocolitis in very-low-birth-weight infants. Pediatrics 60:535–538

Editorial responsibility: Steven Raverty, Abbotsford, British Columbia, Canada

Submitted: November 12, 2015; Accepted: May 15, 2016
Proofs received from author(s): July 10, 2016