Journal of Zoo and Wildlife Medicine 28(3): 298–306, 1997

MORTALITY IN CAPTIVE WILD-CAUGHT HORNED PUFFIN CHICKS (*FRATERCULA CORNICULATA*)

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Abstract: Sixteen horned puffin (*Fratercula corniculata*) and six parakeet auklet (*Cyclorrhynchus psittacula*) chicks of various prefledging ages were caught in Alaska and transported to the North Carolina Zoological Park (USA) in August 1995. Six of the 16 puffin chicks died within a 5-day period beginning 2 days after their arrival into quarantine at the zoo. The birds that died were collected at a young age, weighed 45.4–65.7 g, and had been fed a diet of thawed frozen ocean silversides (*Atherinidae*) that was not supplemented with vitamins. Clinical signs were nonspecific, and gross necropsies, insecticide toxicology screens, and bacterial cultures were unremarkable. Microscopic examination of tissues from five of the six birds showed myocardial necrosis and degeneration suggestive of vitamin E deficiency and intestinal protozoa resembling Microsporidia. The mortality pattern and histopathologic lesions observed in this case support the use of selective age capture and vitamin supplementation for wild alcid chick collection.

Key words: Horned puffin, *Fratercula corniculata*, Alcidae, vitamin E deficiency, Microsporidia.

INTRODUCTION

Alcid exhibits are popular and educational in zoological parks. The low number of horned puffins reproducing successfully in captivity[16,17] makes it necessary to capture wild birds for these displays. The best time to capture chicks is from 7 days of age to fledging because chicks adapt to captivity more readily than do older birds.[3,10,15,21] Egg incubation can be difficult, and younger chicks experience high mortality rates.[35] In this case report, we describe the clinical history and pathologic findings in a group of wild-caught horned puffin chicks (*Fratercula corniculata*) with a 37.5% (6/16) mortality rate and lesions suggestive of vitamin E deficiency.

CASE REPORT

During 10 days in August 1995, a team from the North Carolina Zoological Park (USA) collected 16 horned puffin chicks and six parakeet auklet (*Cyclorrhynchus psittacula*) chicks at Savoonga on St. Lawrence Island, Alaska (USA). Puffins were collected during the first half of the trip and fell into two weight groups: 45.4–65.7 g (n = 8), including 2 eggs that pipped shortly after collection, and 90.1–164.5 g (n = 8). Auklets were collected in the second half of the trip and weighed 126.9–179.1 g. After collection, each chick was placed into an individual compartment (30 × 25 × 20 cm) in a larger peg-board crate. The compartments were lined with brown paper towels and sphagnum moss that were changed every 5 days. A low-wattage bulb provided illumination during feeding periods in the room where the birds were housed. Each bird was handled for approximately 2–4 min s.i.d. for weighing. The chicks were hand-fed t.i.d.–q.i.d. with thawed frozen ocean silversides (Atherinidae) that had been brought on the trip on dry ice. Feeding started within 6 hr of capture. Chicks were fed sufficient food to support a growth rate of approximately 10% of the body weight/day. Auklets received thawed frozen krill (*Euphausia*; greater than 50% of diet) in addition to silversides. Puffin chicks weighing more than 90 g were given a single dose of one-quarter tablet (one tablet/230 g fish fed) of a mixed vitamin supplement (Mazuri®, Vita-Zu® Bird Tablet 5M25, PMI Feeds, Inc., St. Louis, Missouri 63144, USA) 4 days prior to transport (Table 1). Puffin chicks weighing under 90 g and the parakeet auklet chicks did not receive vitamin therapy because of concern about oversupplementation. All chicks were given oral itraconazole (Sporanox, Janssen Pharmaceutica Inc., Titusville, New Jersey 08560, USA) at a dosage of 1 mg/100 g body weight s.i.d., beginning 3 days prior to transport, as a prophylactic measure against fungal diseases. The birds were transported from Alaska to North Carolina in a chilled cargo compartment of a private plane for 21 hr including one layover in San Francisco, California (USA). All birds were fed twice during the trip including once during the layover period. The alcids were transported from the airport to the North Carolina Zoo in an air-conditioned van. On arrival at the zoo, each bird was placed

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Table 1. Contents (per tablet) of the vitamin supplement (Mazuri® Vita-Zu® Bird Tablet 5M25) given to puffin chicks weighing over 90 g.

Ingredient	Amount
Vitamin A	1,650 IU
Vitamin E	25 IU
Vitamin C	25 mg
Thiamine mononitrate	20 mg
Pantothenic acid	1.5 mg
Riboflavin	1.5 mg
Pyridoxine	1.5 mg
Folic acid	50 μg
Biotin	25 μg

into an individual sky kennel (30 × 37.5 × 52.5 cm) with landscape cloth covering the door and sides of the kennel to lower the amount of light within the kennel. Sky kennels were kept in a cold room (10°C) with a 14-hr daily light period for a 30-day quarantine.

Six of the 16 puffin chicks died within a 5-day period beginning 2 days after their arrival into quarantine. The affected birds were in the lower body weight group that had not been supplemented with vitamins. No problem was encountered with the puffin chicks in the heavier body weight group nor with the parakeet auklet chicks. In the six chicks that died, clinical signs, observed on the day of death or in the 24-hr preceding death, ranged from none (1/6) to weight loss (4/6), regurgitation (3/6), anorexia (2/6), or pale mucous membranes (1/6). One surviving chick in the lower weight group showed similar clinical signs and received the same treatment as the chicks that died. Treatment for birds showing clinical signs included enrofloxicin (Baytril®, Miles Animal Health, Shawnee Mission, Kansas 66201, USA) 10 mg/kg p.o. b.i.d., adding water to the fish fed, or tube feeding with a commercially prepared liquid diet (Emeraid II℠, Lafeber Company, Odell, Illinois 60460, USA). The first chick that showed signs of regurgitation was examined endoscopically to the level of the proventriculus and no lesion was observed. The sixth bird to die had pale mucous membranes and was found to be anemic, with a packed cell volume of 22%. As a precaution, itraconazole was discontinued for all puffins on the fifth day of quarantine to eliminate the possibility of toxicity or untoward drug reaction.

Necropsies were performed on all six chicks that died, and samples of heart, lung, air sac, kidney, liver, spleen, proventriculus, ventriculus, intestine, ceca, bursa of Fabricus, skeletal muscle, skin, and brain were fixed in 10% neutral buffered formalin. Specimens were then paraffin embedded, sectioned at 5 μm, stained with hematoxylin and eosin, and examined microscopically. All birds had adequate body fat. Gross abnormalities included enlarged and mottled yellow-red livers (3/6), moderately reduced pectoral muscle mass (2/6), hemorrhages in the lungs with blood in the airways (2/6), watery to mucoid diarrhea (2/6), and pale mucous membranes (1/6). Toxicology screens of the proventricular ingesta of two birds were negative for carbamate and organophosphorus insecticides. General aerobic bacterial culture of lung, liver, and intestinal contents from two birds grew *Staphylococcus* sp. and *Enterococcus* sp., which were considered contaminants, and large numbers of *Escherichia coli* from small intestinal samples only. No *Salmonella* sp. was isolated.

Gross necropsy and histopathologic lesions in the six puffin chicks are summarized in Table 2. Microscopically, the first bird to die had small pulmonary hemorrhages and diffuse bursal lymphoid depletion and necrosis and no other lesion. Multifocal to diffuse myocardial degeneration and necrosis (Fig. 1) were present in the other five birds. Lesions were more severe in birds that survived a longer period of time. The cardiac lesions were characterized by moderate to large pale regions of cardiac muscle cells with swollen, pale, and occasionally fragmented hyaline eosinophilic cytoplasm and pyknotic or karyorrhectic nuclei. Degenerating cardiac muscle cells containing vacuolated cytoplasm were also present (Fig. 2). Cells interpreted as regenerating cardiac muscle cells, characterized by scant basophilic cytoplasm and large vesicular nuclei, were present in low numbers in some of the affected sites. Mineralization was absent from all areas examined using standard stains.

Enteritis and typhlitis were present in five of six birds. Intestinal lesions were characterized by blunt villi, occasionally with villus tip necrosis, and a moderate to marked infiltrate of heterophils with fewer numbers of lymphocytes, plasma cells, and macrophages in the lamina propria. Sloughed enterocytes mixed with heterophils, cell debris, and numerous colonies of short bacterial rods filled the lumen of the ceca and ileum. Along the brush border of the villi and within the crypts in the ileum and ceca of five birds were aggregates of small (1–2 μm), round to oval, basophilic organisms. These organisms stained positively with a Brown-Brenn tissue gram stain, Warthin-Starry stain, and a modified trichrome[32] stain and resembled microsporidia morphologically (Fig. 3).

Multifocal to bridging centrilobular hepatic ne-

Table 2. Summary of abnormalities found in the gross necropsy and histopathologic examination of the horned puffin chicks (puffin chick number refers to order in which birds died).

Lesion	Puffin chick[a]					
	1	2	3	4	5	6
Gross abnormalities						
Enlarged and mottled liver	−	−	++	+	+++	−
Lung hemorrhage	+	−	−	−	+	−
Reduced pectoral muscle mass	−	+	−	−	++	−
Pale mucous membranes	−	+	−	−	−	−
Diarrhea	−	−	−	+	+	−
Histopathology abnormalities						
Myocardial degeneration and necrosis	−	++	++	++	++	+++
Enteritis and typhlitis	−	++	++	++	+++	+
Microsporidia	−	+	+	+	+	+
Hepatic necrosis and degeneration	−	−	++	+++	+++	+
Bursal lymphoid depletion	+	−	−	+	+	+

[a] −, not detected; +, mild; ++, moderate, +++, severe.

crosis and hepatocellular vacuolar degeneration (Fig. 4) ranging from mild to severe were present in four birds. The areas of necrosis consisted of swollen hepatocytes with amorphous eosinophilic cytoplasm and, in some areas, pyknotic or karyorrhectic nuclei mixed with cellular debris (Fig. 5). Some areas of the liver contained intact hepatocytes with large, clear, sharply bordered vacuoles, presumed to contain lipid. Lymphoid depletion and absent to mild lymphoid necrosis in the bursa of Fabricus (Fig. 6), without evidence of inflammation or a specific etiology, were found in four birds.

DISCUSSION

The cardiac lesions found in five of the six birds that died in the short period following arrival at the North Carolina Zoological Park were suggestive of toxicity, deficiency, capture stress, or possibly a combination of these factors. The diet and vitamin supplementation history suggests that vitamin E de-

Figure 1. Histologic section of myocardium from the left ventricle of a horned puffin demonstrating multifocal to diffuse myocardial degeneration and necrosis. H&E stain, × 13.2.

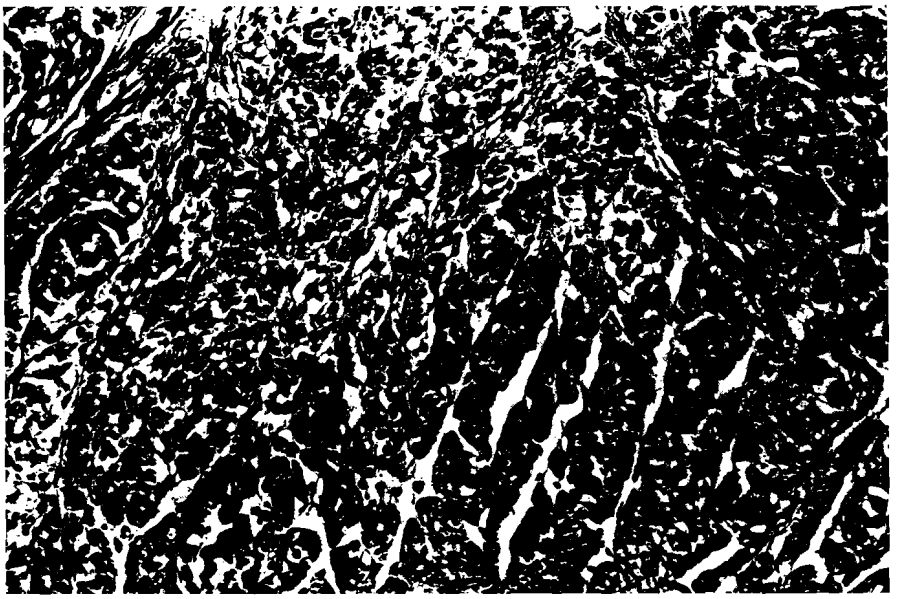

Figure 2. Histologic section of myocardium from the left ventricle of a horned puffin demonstrating myocardial degeneration. In regions, myocytes are swollen and degenerate with pale hyaline eosinophilic cytoplasm and pyknotic nuclei (arrow). H&E stain, ×66.

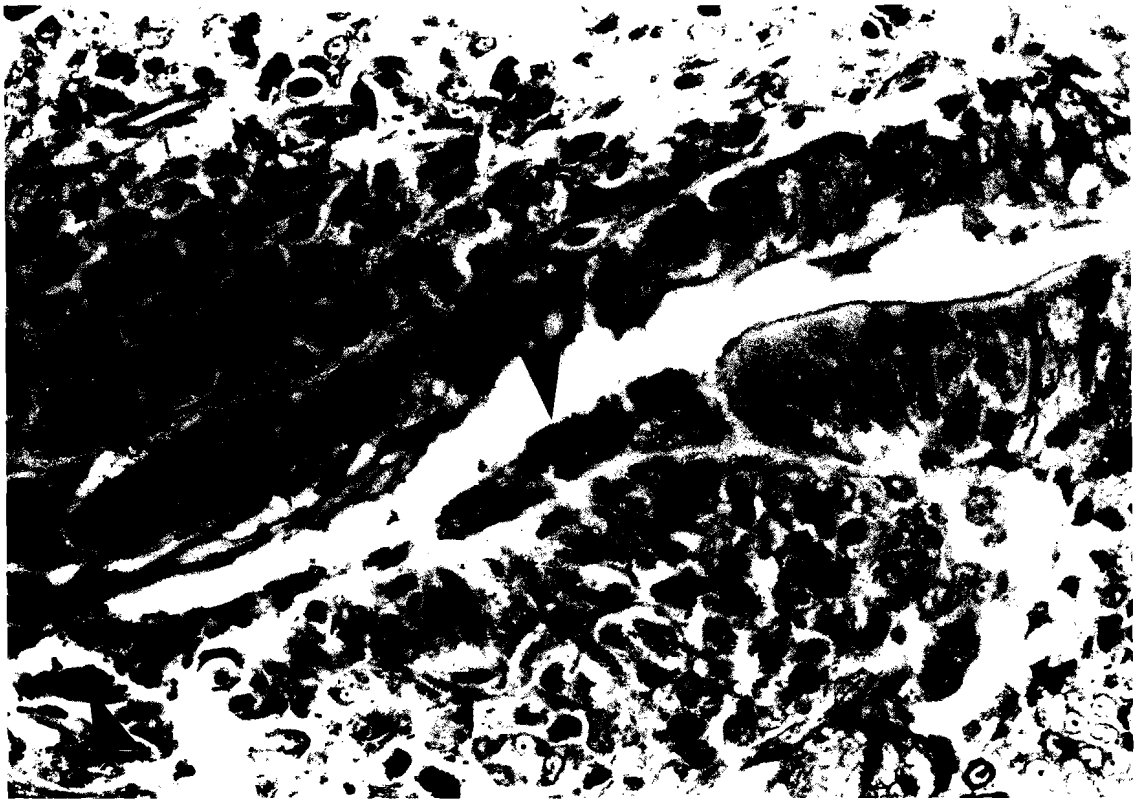

Figure 3. Histologic section of cecum of a horned puffin demonstrating typhlitis and mucosal epithelial necrosis. Note inflammatory cells in the lamina propria and presumptive microsporidia lining the epithelium (arrow). Giemsa stain, ×500.

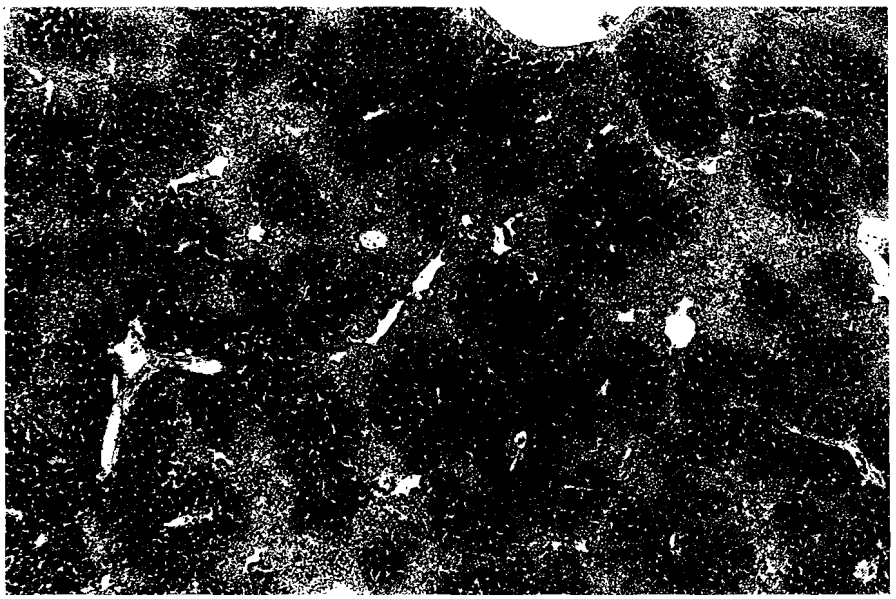

Figure 4. Histologic section of liver of a horned puffin demonstrating multifocal to bridging centrilobular necrosis and hepatocellular vacuolar degeneration. H&E stain, ×10.

ficiency could be the cause of the cardiac lesions. Vitamin E functions as a major scavenger of free radicals and peroxides and works in concert with glutathione peroxidase, a selenium-dependent intracellular enzyme, to reduce peroxides and protect the cell from oxidative damage.[11,19] In the presence of inflammation (enteritis, typhlitis), antioxidants such as vitamin E are important in modulating the inflammatory reaction and scavenging free radicals produced by inflammatory cells and must be provided in the diet.[5] Vitamin E deficiency in poultry causes acute hyaline degeneration of skeletal mus-

Figure 5. Histologic section of liver of a horned puffin demonstrating normal hepatocytes, bile duct epithelium (open arrow), and centrilobular hepatocellular necrosis (closed arrow). Necrotic areas consist of swollen hepatocytes with pyknotic nuclei and amorphous eosinophilic cytoplasm mixed with cellular debris. H&E stain, ×100.

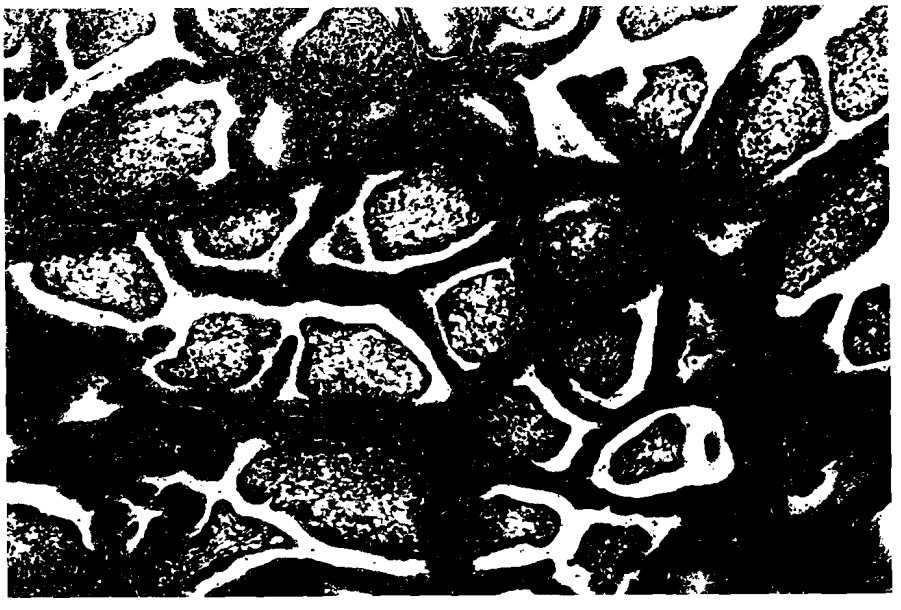

Figure 6. Histologic section of the bursa of Fabricus of a horned puffin showing lymphoid depletion and mild necrosis of follicles. H&E stain, ×25.

cle cells followed by necrosis and fragmentation. Chronic lesions may be characterized by attempts at repair and regeneration, with pronounced proliferation of cell nuclei and, eventually, some fibrosis,[1] as was observed in the myocardium of some of the affected puffin chicks. The natural diet of the horned puffin includes a variety of small marine fish and invertebrates.[34] Many marine fish (e.g., silversides) contain high concentrations of polyunsaturated fatty acids that readily degrade to produce free radicals and peroxides. Free radicals and peroxides accumulate in tissues of piscivorous species and can cause oxidative damage if tissue vitamin E concentrations are low.[12] Selenium is present in both the lipid and nonlipid portions of fish[12,22] and is not subject to degradation. Steatitis and myocardial degeneration have been found in other species of birds fed high-fat fish diets.[6,8,26,27] The myocardial degeneration, necrosis, and regeneration without evidence of concurrent inflammation in these puffin chicks, as well as the hepatic necrosis and anemia, are similar to lesions reported previously for vitamin E deficiency.[1,19] The hepatic centrilobular necrosis seen in these puffins was most likely produced by decreased cardiac output or cardiac insufficiency resulting in secondary centrilobular hypoxemia. Steatitis was not noted in any of the puffins, which could be a reflection of the acute nature of the incident.

Similar lesions may be observed with toxic myopathies (i.e., ionophores, *Cassia* spp., gossypol); however, the lack of inflammation or mineralization characteristic of other toxicities and the lack of a history of exposure to these toxins make them unlikely causes of the myocardial lesions.[18,20] A deficiency in other antioxidants or compounds, such as vitamin C, vitamin A, or selenium, may have contributed to the abnormalities, although this is less likely because of the character of the histopathologic abnormalities (cardiac muscle degeneration, necrosis) and the composition of the diet fed.

A similar presentation of heart lesions leading to hepatic centilobular necrosis has been seen in animals with subacute capture myopathy syndrome or capture stress leading to cardiac distress and occasional collapse.[9] Stress in animals has been defined as stimuli that produce a change or stress response.[4] Although husbandry and management factors were held as constant as possible, certain aspects of the management of the alcids did change, including noises from air travel, transfer from the peg-board crate into larger sky kennels for the quarantine period, and minor environmental temperature fluctuations during air and ground transport. These and other factors could have stressed the birds and contributed to the onset of disease.

The bursa of Fabricus was examined microscopically in the six dead puffins, and evidence of lymphocyte and lymphoid follicle depletion and mild necrosis was found in four birds. Vitamin E- and selenium-deficient diets have been shown to cause lymphoid depletion.[23] In the chicken, the bursa is

known to decrease in size and begin normal involution by 8–13 wk of age.[13] Although little is known about the normal regression of the bursa in alcids, the birds in this case report were approximately 4–6 wk old, and normal regression of the bursa would not have been expected. The epithelial layer surrounding the bursa lymphoid follicles in the puffins remained intact, with no evidence of the epithelial metaplasia that would be expected with dietary deficiency of vitamin A, a potential cause of bursal lymphoid regression in chickens.[2] Massive amounts of glucocorticoids are known to be lympholytic to avian bursal lymphoid cells.[14] The loss of lymphocytes seen in the puffin chicks could have been a result of excess glucocorticoid production caused by the presence of stress factors (environmental, dietary, handling, infectious). However, all birds were handled in a similar manner and were subject to the same stresses.

The protozoanlike organisms most closely resembling microsporidia found in the lower intestinal tract of five of six birds necropsied were not observed in any other tissue. Microsporidia are obligate intracellular protozoans that reproduce by spore formation. The organisms observed in the intestine appeared to be extracellular and most likely represented the transmission spore stage of the microsporidia. Transmission is usually by the oral route, although transplacental transmission has been documented in mammals.[7] Microsporidia have not been documented previously in alcids but have been found in lovebirds (*Agapornis* spp.), where they have been associated with a pattern of hepatic necrosis similar to that seen in the affected alcids.[28,30] No protozoal organism was observed in the livers of these puffin chicks with hematoxylin and eosin, giemsa, Brown-Brenn gram, or Warthin-Starry stains. Microsporidia stain positively with a silver stain and are the only protozoa that stain Gram positive.[7] A modified trichrome stain developed for easier identification of microsporidia infections in human patients[32] was used for confirmation of the presence of the organism in the puffin intestinal tract. The organisms were consistently associated with intestinal mucosal inflammation and necrosis varying from mild to severe. Large colonies of mixed bacteria were associated with these organisms, and bacterial culture of two chicks grew *E. coli* from the ileal and cecal contents. These coliforms may have contributed to the enteritis and typhlitis observed. Microsporidia have an extensive host range, including invertebrates and the five classes of vertebrates. They are of increasing importance in both human and veterinary medicine because of their potential to infect and cause disease in immunocompromised hosts.[7,33] Infection with microsporidia can be inapparent but also can be associated with systemic disease. The role microsporidia played in the pathogenesis of the enteritis and typhlitis or in the myocardial lesions observed in the puffins is unknown. The microsporidia infection could have been an incidental finding, an opportunistic infection in immunocompromised chicks, or could have added to the stress of the birds, contributing to lymphoid depletion. If the surviving wild-caught puffins or parakeet auklets of this report are infected with microsporidia or if this organism has significance in a healthy population is not known. Screening of fecal smears[32] of the puffin and auklet group did not reveal microsporidia.

The affected puffin chicks were in the lower weight group at capture and were fed a thawed frozen diet of high lipid content without vitamin supplementation. Because of the various deficiency problems associated with feeding piscivorous birds, specific vitamin supplementation regimes have been formulated[24] and have been used in newly captured alcids with varying success.[10,21,25,35,36] In this case, two birds of the low weight group that did not receive vitamin supplements prior to transport survived, one of which showed clinical signs. Whether these two birds developed lesions similar to those seen in the puffin chicks that died or will have evidence of hepatic or myocardial lesions in the future is not known.

None of the unsupplemented parakeet auklet chicks showed signs of clinical disease and none died. The parakeet auklet chicks were older than the puffins when captured, grew more slowly, and were fed a different diet. A major part of the diet of the auklets was krill, which has a higher content of vitamin A and E and contains fewer polyunsaturated fats[12,29] than most teleost fishes and therefore is less susceptible to fatty acid degradation and the production of free radicals and peroxides. Unfortunately, plasma or tissue vitamin levels could not be evaluated in the chicks because of their small size and the lack of sufficient tissues for analysis.

The mortality and the gross and microscopic lesions found in this group of wild-caught puffin chicks support earlier suppositions that birds intended for captivity should be captured above a minimum age using body weight, wing length, or pin feather size as guides.[3,15,21] The lesions found in the puffin chicks most likely represented an unusual presentation of vitamin E deficiency, although no definitive testing was done to confirm this hypothesis. However, dietary supplementation with multivitamin preparations that include vitamin E of

birds fed fish diets[24] is a warranted precaution in future collecting trips if frozen fish diets are to be used.[12,31] The presumptive diagnosis of intestinal microsporida was made with light microscopic examination and stain reactions. Tissues were insufficiently preserved for the use of electron microscopy to definitively diagnose microsporidiosis. Additionally, we recommend the use of sanitation protocols that minimize fecal–oral contamination in order to decrease the potential for transmission of microsporidia and other infectious organisms.

Acknowledgments: We thank Drs. F. Daldorf and D. Meuten for their assistance with histopathologic interpretation and Dr. H. Stout for thoughtful review of the manuscript.

LITERATURE CITED

1. Austic, R. E., and M. L. Scott. 1991. Nutritional diseases. *In:* Calnek, B. W., H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. (eds.). Diseases of Poultry, 9th ed. Iowa State University Press, Ames, Iowa. Pp. 45–71.

2. Bang, B. G., F. B. Bang, and M. A. Foard. 1972. Lymphocyte depression induced in chickens on diets deficient in vitamin A and other compounds. Am. J. Pathol. 68: 147–162.

3. Bohmke, B. W. 1983. Collecting alcids in Iceland. Reg. Proc. Conf. Am. Assoc. Zool. Parks Aquarium, Evansville, Indiana. Pp. 447–449.

4. Breazile, J. E. 1987. Physiologic basis and consequences of distress in animals. J. Am. Vet. Med. Assoc. 191: 1212–1215.

5. Breazile, J. E. 1988. The physiology of stress and its relationship to mechanisms of disease and therapeutics. Vet. Clin. North Am. Food Anim. Pract. 4: 441–480.

6. Campbell, G., and R. J. Montali. 1980. Myodegeneration in captive brown pelican attributed to vitamin E deficiency. J. Zoo Wildl. Med. 11: 35–40.

7. Canning, E. U. 1986. The Microsporidia of Vertebrates. Academic Press, Inc., Orlando, Florida.

8. Carpenter, J. W., J. W. Spann, and M. N. Novilla. 1979. Diet-related die-off of captive black-crowned night herons. Proc. Am. Assoc. Zoo Vet. 1979: 51–55.

9. Chalmers, G. A., and M. W. Barrett. 1982. Capture myopathy. *In:* Hoff, G. L., and J. W. Davis (eds.). Noninfectious Diseases of Wildlife. Iowa State University Press, Ames, Iowa. Pp. 84–94.

10. Conway, W. G., J. Bell, D. Bruning, and E. Dolensek. 1977. Care and breeding of puffins and murres at the New York Zoological Park. Int. Zoo Yearb. 17: 173–176.

11. Dierenfeld, E. S. 1989. Vitamin E deficiency in zoo reptiles, birds, and ungulates. J. Zoo Wildl. Med. 20: 3–11.

12. Geraci, J. R., and D. J. St. Aubin. 1980. Nutritional disorders of captive fish-eating animals. *In:* Montali, R. J., and G. Migaki (eds.). Comparative Pathology of Zoo Animals. Smithsonian Institution, Washington, D.C. Pp. 41–49.

13. Glick, B. 1956. Normal growth of the bursa of Fabricus in chickens. Poult. Sci. 35: 843–851.

14. Glick, B. 1967. Antibody and gland studies in cortisone and ACTH-injected birds. J. Immunol. 98: 1076–1084.

15. Gunther, M. R. 1988. Captive management of the common puffin at the National Aquarium in Baltimore. Reg. Proc. Conf. Am. Assoc. Zool. Parks Aquarium, Salisbury, Maryland. Pp. 692–695.

16. Gunther, M. R. 1991. The status of alcids in North American zoological institutions. Annu. Proc. Conf. Am. Assoc. Zool. Parks Aquarium. Pp. 231–237.

17. Gunther, M. R. 1994. Alcids in North American zoos and aquaria. Int. Zoo Yearb. 33: 136–141.

18. Hanrahan, L. A., D. E. Corrier, and S. A. Nagi. 1981. Monensin toxicosis in broiler chickens. Vet. Pathol. 18: 665–671.

19. Jones, T. C., and R. D. Hunt. 1983. Hypovitaminosis E. *In:* Jones, T. C., and R. D. Hunt (eds.). Veterinary Pathology, 5th ed. Lea and Febiger, Philadelphia, Pennsylvania. Pp. 1044–1049.

20. Julian, R. J. 1991. Poisons and toxins. *In:* Calnek, B. W., H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. (eds.). Diseases of Poultry, 9th ed. Iowa State University Press, Ames, Iowa. Pp. 863–884.

21. Kress, S. W. 1978. Establishing Atlantic puffins at a former breeding site. *In:* Temple, S.A. (ed.). Endangered Birds: Management Techniques for Preserving Threatened Species. University of Wisconsin Press, Madison, Wisconsin. Pp. 373–377.

22. Lunde, G. 1970. Analysis of arsenic and selenium in marine raw materials. J. Sci. Food Agric. 21: 242–247.

23. Marsh, J. A., G. F. Combs, Jr., M. E. Whitacre, and R. R. Dietert. 1986. Effect of selenium and vitamin E dietary deficiencies and chick lymphoid organ development. Proc. Soc. Exp. Biol. Med. 182: 425–436.

24. Mejeur, J. H., E. S. Dierenfeld, and J. A. Murtaugh. 1988. Development of a vitamin supplement for puffins and other alcids. Reg. Proc. Conf. Am. Assoc. Zool. Parks Aquarium, Salisbury, Maryland. Pp. 696–700.

25. Monroe, A. 1991. Tufted puffin reproduction. Proc. Conf. Am. Assoc. Zool. Parks Aquarium. Pp. 245–251.

26. Nichols, D. K., V. L. Campbell, and R. J. Montali. 1986. Pansteatitis in great blue herons. J. Am. Vet. Med. Assoc. 189: 1110–1112.

27. Nichols, D. K., and R. J. Montali. 1987. Vitamin E deficiency in captive and wild piscivorous birds. Proc. First Int. Conf. Zool. Avian Med., Turtle Bay, Hawaii. Pp. 419–421.

28. Novilla, M. N., and R. P. Kwapien. 1977. Microsporidian infection in the pied peached-faced lovebird. Avian Dis. 22: 198–204.

29. Pennino, M., E. S. Dierenfeld, and J. L. Behler. 1991. Retinol, α-tocopherol and proximate nutrient composition of invertebrates used as feed. Int. Zoo Yearb. 30: 143–149.

30. Powell, S., K. Tang, F. Chandler, D. Parks, and C.

Hood. 1989. Microsporidiosis in a lovebird. J. Vet. Diagn. Invest. 1: 69–71.

31. Stoskopf, M. K. 1986. Feeding picivorous birds, a review. Proc. Am. Assoc. Zoo Vet. 1986: 68–87.

32. Weber, R., R. T. Bryan, R. L. Owen, C. M. Wilcox, L. Gorelkin, and G. V. Visvesvara. 1992. Improved light-microscopic detection of microsporidia spores in stool and duodenal aspirates. N. Engl. J. Med. 326: 161–166.

33. Weber, R., R. T. Bryan, D. A. Schwartz, and R. L. Owen. 1994. Human microsporidial infections. Clin. Microbiol. Rev. 7: 426–461.

34. Wehle, D. H. S. 1983. The food, feeding, and development of young tufted and horned puffins in Alaska. Condor 85: 427–442.

35. Wikenhauser, C. 1981. The hand rearing and captive management of common puffins (*Fratercula artica*). Reg. Proc. Conf. Am. Assoc. Zool. Parks Aquarium, Peoria, Illinois. Pp. 302–306.

36. Zombeck, D. J. 1991. Hand-rearing wild-caught tufted puffin chicks at Sea World of Florida. Annu. Proc. Conf. Am. Assoc. Zool. Parks Aquarium. Pp. 238–244.

Received for publication 22 April 1996