collected under permit from the Canadian Wildlife Service and with permission from the Government of Newfoundland and Labrador. This study was supported by the Canadian Wildlife Service and the Departments of Veterinary Microbiology and Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan.

LITERATURE CITED

BLANK, H., C. DAVIS, AND C. COLLINS. 1970. Electron microscopy for the diagnosis of cutaneous viral infection. British Journal of Dermatology 83: 69–80.

CREUTZ, G., AND C. GOTTSCHALK. 1969. Endoparasitenbefall bei Lachmöwen in Abhängigkeit vom Alter. Angewandte Parasitologie 10: 80–91.

CURRENT, W. L., AND P. L. LONG. 1983. Development of human and calf *Cryptosporidium* in chicken embryos. The Journal of Infectious Diseases 148: 1108–1113.

DORAN, D. J. 1978. The life cycle of *Eimeria dispersa* Tyzzer 1929 from the turkey in gallinaceous birds. The Journal of Parasitology 64: 882–885.

GAJADHAR, A. A., R. J. CAWTHORN, AND D. J. RAINNIE. 1982. Experimental studies on the life cycle of a renal coccidium of lesser snow geese (*Anser c. caerulescens*). Canadian Journal of Zoology 60: 2085–2092.

——, D. J. RAINNIE, AND R. J. CAWTHORN. 1986. Description of the goose coccidium *Eimeria stigmosa* (Klimes, 1963), with evidence of intranuclear development. The Journal of Parasitology 72: 588–594.

——, AND P. H. G. STOCKDALE. 1986. Ultrastructural studies of microgametogenesis and macrogametogenesis of *Eimeria truncata* of the lesser snow goose. Journal of Protozoology 33: 345–351.

——, G. WOBESER, AND P. H. G. STOCKDALE. 1983. Coccidia of domestic and wild waterfowl (Anseriformes). Canadian Journal of Zoology 61: 1–24.

LEIGHTON, F. A., AND A. A. GAJADHAR. 1986. *Eimeria fraterculae* sp. n. in the kidneys of Atlantic puffins (*Fratercula arctica*) from Newfoundland, Canada: Species description and lesions. Journal of Wildlife Diseases 22: 520–526.

LEVINE, N. D. 1985. Veterinary protozoology, 1st ed. Iowa State University Press, Ames, Iowa, 414 pp.

MONTGOMERY, R. D., M. N. NOVILLA, AND R. B. SHILLINGER. 1978. Renal coccidiosis caused by *Eimeria gaviae* n. sp. in a common loon (*Gavia immer*). Avian Diseases 22: 809–814.

MUNDAY, B. L., R. W. MASON, R. J. H. WELLS, AND J. H. ARUNDEL. 1971. Further studies on "limey disease" of Tasmanian mutton birds (*Puffinus tenuirostris*). Journal of Wildlife Diseases 7: 126–129.

OBENDORF, D. L., AND K. McCOLL. 1980. Mortality in little penguins (*Eudyptula minor*) along the coast of Victoria, Australia. Journal of Wildlife Diseases 16: 251–259.

OVERSTREET, R. M., W. E. HAWKINS, AND J. W. FOURNIE. 1984. The coccidian genus *Calyptospora* n. g. and family Calyptosporidae n. fam. (Apicomplexa), with members infecting primarily fishes. Journal of Protozoology 31: 332–339.

PELLERDY, L. P. 1974. Coccidia and coccidiosis, 2nd ed. Verlag Paul Parey, Berlin, Federal Republic of Germany, 959 pp.

SHEFFIELD, C. A., A. A. MARCHIONDO, AND B. MUELLER. 1979. Scanning and transmission electron microscopy of the oocyst wall of *Isospora lacazei*. Zeitschrift für Parasitenkunde 59: 219–225.

THOMPSON, E. J., AND I. C. A. WRIGHT. 1978. Coccidiosis in kiwis. New Zealand Veterinary Journal 26: 167.

Received for publication 26 August 1987.

4747

SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 24(3), 1988, pp. 547–550
© Wildlife Disease Association 1988

Vitamin E in Captive and Wild Black Rhinoceros (*Diceros bicornis*)

Ellen S. Dierenfeld, Raoul du Toit, and R. Eric Miller, Animal Health Center, New York Zoological Society, Bronx, New York 10460, USA; IUCN African Elephant and Rhino Specialist Group, Harare, Zimbabwe; St. Louis Zoological Park, St. Louis, Missouri 63110, USA

ABSTRACT: The mean plasma level of α-tocopherol (vitamin E) measured in 31 free-ranging black rhinoceros (*Diceros bicornis*) was significantly higher ($P < 0.001$) than that in 11 captive animals ($\bar{x} \pm SE = 0.77 \pm 0.05$ and 0.18 ± 0.03 μg/ml, respectively). Vitamin E status may influence the health of captive black rhinoceros; in particular, it may be linked to hemolytic anemia commonly observed in these animals in captivity.

Key words: Vitamin E, nutrition, herbivore, black rhinoceros, *Diceros bicornis*, hemolytic anemia, field study.

Deficiency of α-tocopherol (vitamin E) has been reported in a number of captive herbivores (Dolensek and Combs, 1985). Lesions observed in captive hoofstock and primates include muscular dystrophy (Liu et al., 1982), neuronal degeneration (Liu et al., 1983), cardiomyopathy (Liu et al., 1984) and anemia (Dinning and Day, 1957). Pathology consistent with vitamin E deficiency also has been reported in cases of capture myopathy in wild populations (Basson and Hofmeyr, 1973). However, capture myopathy has been recorded in species with apparently normal vitamin E levels (Spraker, 1980) and several factors including muscular exertion, stress and nutrient deficiencies are probably involved in the etiology of this disease. Nonetheless, comparisons between vitamin E levels in captive species and their free-ranging counterparts are rare, making it difficult to assess vitamin E status in either population even when circulating levels are known. This report documents preliminary data concerning the vitamin E status of black rhinoceros (*Diceros bicornis*) and discusses the possible link of vitamin E with hemolytic anemia in these large herbivores.

Blood samples from 31 black rhinoceros were obtained between 3 June and 28 July 1986 during a translocation operation centered in the Zambezi Valley (16°00'S, 29°30'E), Zimbabwe. An additional 16 blood samples were taken from 12 captive animals at various zoos in the United States between 1984 and 1987 (St. Louis Zoological Park, Forest Park, St. Louis, Missouri 63110, USA, $n = 7$; Denver Zoological Gardens, City Park, Denver, Colorado 80205, USA, $n = 3$; Lee Richardson Zoo, Box 499, Garden City, Kansas 67846, USA, $n = 2$; Zoo Atlanta, 800 Cherokee Avenue SE, Atlanta, Georgia 30315, USA, $n = 1$; Cheyenne Mountain Zoological Park, Box 158, Colorado Springs, Colorado 80901, USA, $n = 1$; Detroit Zoological Park, Box 39, Royal Oak, Michigan 48068, USA, $n = 1$; and Los Angeles Zoo, 5333 Zoo Drive, Los Angeles, California 90027, USA, $n = 1$). Although no special precautions were taken to protect blood samples from sunlight, potential exposure times were usually < 5 min.

Plasma was separated by centrifugation and frozen immediately. Following ethanol precipitation and hexane extraction by the procedures described in Dierenfeld and Dolensek (1988), α-tocopherol (vitamin E) content was determined using high performance liquid chromatography. A Series 400 chromatograph (Perkin-Elmer, Inc., Norwalk, Connecticut 06859, USA) equipped with a 30-cm C-18 column was used for separation; the vitamin E peak was monitored by a Perkin-Elmer fluores-

cence detector (Model LS-1), and peak areas on the chromatograph were analyzed with a Perkin-Elmer Model LCI-100 data processor. Serum cholesterol was measured on the Technicon RA-1000 (Technicon Instrumentations Corporation, Tarrytown, New York 10591, USA). Means were compared using Student's *t*-test (Snedecor and Cochran, 1967); statistical significance was determined at $P = 0.05$.

Circulating levels of vitamin E in the wild black rhinoceros ($\bar{x} = 0.77 \pm 0.05$ μg/ml) were significantly ($P < 0.001$) higher than those seen in 11 captive animals ($\bar{x} = 0.18 \pm 0.03$ μg/ml) fed diets unsupplemented with vitamin E. One 212-kg 7-mo-old female, experimentally supplemented with 1000 IU of d,l α-tocopheryl acetate orally since 1 mo of age, was not included in the mean for the zoo animals. Her plasma vitamin E level was 3.87 ± 0.05 μg/ml. Differences between sexes were not significant ($P > 0.05$).

Cholesterol levels measured in zoo (70.10 ± 10.47 mg/dl, $n = 10$) and free-ranging (71.71 ± 6.84 mg/dl, $n = 17$) black rhinoceros were not statistically different, but cholesterol was positively correlated with vitamin E ($r = 0.92$, $P < 0.05$). Vitamin E is a fat soluble vitamin carried in lipid components of the blood; correction for blood lipids (either cholesterol or total lipids) has been suggested to standardize and evaluate vitamin E status within species (Horwitt et al., 1972; Maybew et al., 1987) and provide a basis of comparison among species. Vitamin E:cholesterol ratios (per ml plasma) ranged from 0.1 to 0.4 in captive black rhinoceros, and 0.8 to 2.0 in free-ranging animals.

Free-ranging rhinoceros were immobilized using etorphine hydrochloride (M-99 etorphine, D-M Pharmaceuticals, Rockville, Maryland 20850, USA). All, with the exception of one female with a prolapsed rectum, were assessed to be in excellent physical condition at the time of capture based upon veterinary clinical, hematological and parasite examinations. Medical history of the zoo animals (also immobilized with etorphine hydrochloride) indicates that one rhinoceros (Studbook #161), whose vitamin E level was undetectable (<0.05 μg/ml) in this sample, had previously experienced recurrent bouts of mild hemolytic anemia. Another rhinoceros (Studbook #328), with a plasma α-tocopherol level of 0.22 μg/ml, was in the midst of a hemolytic crisis when this sample was collected. Others appeared clinically normal.

Circulating levels < 1.5 μg/ml α-tocopherol are considered deficient in both cattle and horses (Stowe, 1968; Stuart, 1987); normal levels of the vitamin are generally >3.0 μg/ml. By comparison with domestic livestock, both free-ranging and captive black rhinoceros may be vitamin E deficient.

Vitamin E deficiency is known to influence membrane integrity and cause erythrocyte hemolysis in primates, rats and horses (Stowe, 1968; Bieri and Poukka, 1970; Ausman and Hayes, 1974). Vitamin E deficiency in the black rhinoceros should be considered as one possible etiology for hemolytic anemia in this species. One can speculate that vitamin E deficiency may act primarily as a factor in increased erythrocyte (RBC) membrane fragility with subsequent rupture (hemolysis), and/or increase the susceptibility of the RBC to a number of oxidant stresses (Horwitt, 1968; Stowe, 1968; Tappel, 1972; Tudhope and Hopkins, 1975). Although other factors may be involved in the development of hemolytic anemia, further research is needed to demonstrate these relationships in the black rhinoceros.

Hemolytic anemia has been reported in 15 captive black rhinoceros in zoos in North America, Japan and Europe (Miller and Boever, 1982) and observed in an additional 14 animals (R. E. Miller, unpubl. data). Twenty of these animals died during hemolytic crises. Diets of the zoo animals typically contained large amounts of produce and dried forages, with little or no access to fresh pasture.

By comparison, all wild black rhinoceros sampled in this study were found in prime habitat, with high availability of herbaceous and woody browse species growing on a fertile mix of alluvial and colluvial soils at the base of the Zambezi escarpment. Although levels of vitamin E were not determined in either captive or free-ranging animal diets, domestic livestock fed commercially processed diets and/or hays typically consume less than one-third the amount of α-tocopherol they would if allowed to graze fresh pasture; they often develop vitamin E deficiencies (Mayhew et al., 1987; Stuart, 1987). The lack of information on dietary vitamin E levels is a serious limitation of this study, but it is currently under investigation.

The magnitude of the differences in plasma α-tocopherol levels seen between free-ranging and captive black rhinoceros is almost identical to that found between wild and unsupplemented captive elephants ($\bar{x} = 0.79 \pm 0.05$ and 0.17 ± 0.03 μg/ml α-tocopherol, respectively; U. S. Seal, pers. comm.). Again, information is lacking, but evidence suggests that dietary vitamin E requirements are not being met for many herbivores fed typical zoo diets (Lau et al., 1982, 1983, 1984). Elephants that have been supplemented daily with vitamin E at a level of approximately 2 IU/kg body mass show a mean circulating vitamin E value of 0.6 ± 0.1 μg/ml (Dierenfeld and Dolensek, 1988).

One Indian rhinoceros (*Rhinoceros unicornis*) supplemented with vitamin E at 2 IU/kg body mass daily for 2 yr showed a single plasma α-tocopherol value of 0.5 μg/ml (E. S. Dierenfeld, unpubl. data). The heavily supplemented black rhinoceros described earlier was fed a minimum of 4.5 IU vitamin E per kg body mass per day for 6 mo prior to blood sampling.

White (*Ceratotherium simum*) and Indian rhinoceros are primarily grazers, rather than browsers like the black rhinoceros (Hoppe, 1984). Hemolytic anemia has not been reported for either species in captivity. Dietary requirements for vitamin E may vary between these grazers and the browsing black rhinoceros. Nonetheless, it is suggested that dietary supplementation of α-tocopherol at a minimum of 2.0 to 2.5 IU/kg body mass is a prudent management practice for feeding captive black rhinoceros.

The authors appreciate the efforts of E. Dolensek, New York Zoological Society, New York, New York; D. Mehnick, Columbia University, New York, New York and R. Cambre, Denver Zoological Gardens, Denver, Colorado in obtaining and coordinating samples. R. Moretti and J. Duffelmeyer, Animal Health Center, New York Zoological Society, Bronx, New York, assisted with laboratory analysis. Permission to collect samples from rhinoceros in the Zambezi Valley was given by the Director, Department of National Parks and Wildlife Management, Zimbabwe, and work was facilitated by C. Coetsee and his staff of the Umtshibi Management Unit. Funding for this work was provided by the World Wildlife Fund and Hoffmann-LaRoche, Inc.

LITERATURE CITED

AUSMAN, L. M., AND K. C. HAYES. 1974. Vitamin E deficiency anemia in old and new world monkeys. American Journal of Clinical Nutrition 27: 1148–1154.

BYERS, P. A., AND J. M. HOLMYARD. 1973. Mortalities associated with wildlife capture operations. *In* The capture and care of wild animals, J. Young (ed.). Human and Rousseau, Capetown, Republic of South Africa, pp. 151–160.

BIERI, J. G., AND R. K. H. POUKKA. 1970. In vitro hemolysis as related to rat erythrocyte content of alpha-tocopherol and polyunsaturated fatty acids. Journal of Nutrition 100: 557.

DIERENFELD, E. S., AND E. P. DOLENSEK. 1988. Circulating levels of vitamin E in captive Asian elephants (*Elephas maximus*). Zoo Biology 7: In press.

DINNING, J. S., AND P. L. DAY. 1957. Vitamin E deficiency in the monkey. I. Muscular dystrophy, hematologic changes, and excretion of urinary nitrogenous constituents. Journal of Experimental Medicine 105: 395–403.

DOLENSEK, E. P., AND S. B. COMBS. 1985. Vitamin E deficiency in zoo animals. Proceedings of the Fourth Annual Dr. Scholl Conference on the Nutrition of Captive Wild Animals, Chicago, Illinois. 140 pp.

HOPPE, P. P. 1984. Strategies of digestion in African

herbivores. *In* Herbivore nutrition in the subtropics and tropics, F. M. C. Gilchrist and R. I. Mackie (eds.). The Science Press, Craighall, Republic of South Africa, pp. 229–233.

HORWITT, M. K. 1968. Lipids, alpha tocopherol and erythrocyte hemolysis. Vitamins and Hormones 26: 487–499.

———, C. C. HARVEY, H. C. DAHM, JR., AND M. T. SEARCY. 1972. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Annals of the New York Academy of Science 203: 223–236.

LIU, S. K., E. P. DOLENSEK, C. R. ADAMS, AND J. P. TAPPE. 1983. Myelopathy and vitamin E deficiency in six Mongolian wild horses. Journal of the American Veterinary Medical Association 183: 1266–1272.

———, ———, A. J. HERRON, J. STOVER, AND J. G. DOHERTY. 1982. Myopathy in the nyala. Journal of the American Veterinary Medical Association 181: 1232–1236.

———, ———, J. P. TAPPE, J. STOVER, AND C. R. ADAMS. 1984. Cardiomyopathy associated with vitamin E deficiency in seven gelada baboons. Journal of the American Veterinary Medical Association 185: 1347–1350.

MAYHEW, I. G., C. M. BROWN, H. D. STOWE, A. L. TRAPP, F. J. DERKSEN, AND S. F. CLEMENT. 1987. Equine degenerative myeloencephalopathy: A familial disease of Equidae caused by vitamin E deficiency? Journal of Veterinary Internal Medicine 1: 45–50.

MILLER, R. E., AND W. J. BOEVER. 1982. Fatal hemolytic anemia in the black rhinoceros: Case report and a survey. Journal of the American Veterinary Medical Association 181: 1228–1231.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames, Iowa, 593 pp.

SPRAKER, T. R. 1980. Pathophysiology associated with capture of wild animals. *In* The comparative pathology of zoo animals, R. J. Montali and G. Migaki (eds.). Smithsonian Institution, Washington, D.C., pp. 403–414.

STOWE, H. D. 1968. Alpha tocopherol requirements for equine erythrocyte stability. American Journal of Clinical Nutrition 21: 135–142.

STUART, R. L. 1987. Factors affecting the vitamin E status of beef cattle. Proceedings of the Roche Technical Symposium—The Role of Vitamins on Animal Performance and Immune Response. Daytona Beach, Florida, 80 pp.

TAPPEL, A. L. 1972. Vitamin E and free radical peroxidation in lipids. Annals of the New York Academy of Science 203: 12–28.

TUDHOPE, G. R., AND J. HOPKINS. 1975. Lipid peroxidation in human erythrocytes in tocopherol deficiency. Acta Haematologica 53: 98–104.

Received for publication 21 August 1987.

Journal of Wildlife Diseases, 24(3), 1988, pp. 551–554
© Wildlife Disease Association 1988

Preliminary Evaluation of Praziquantel Against Metacercariae of *Nanophyetus salmincola* in Chinook Salmon (*Oncorhynchus tshawytscha*)

William J. Foreyt[1] and John R. Gorham,[2] [1] Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164, USA; [2] Animal Disease Unit, Washington State University, Pullman, Washington 99164, USA

ABSTRACT: Praziquantel at dosages of 10, 20 or 100 mg/kg of body weight was evaluated against metacercariae of *Nanophyetus salmincola* in chinook salmon (*Oncorhynchus tshawytscha*). Ten salmon were used in each of four treated groups and 10 salmon were nontreated controls. Three wk after treatment, viability of metacercariae was determined by histologic evaluation and by feeding the salmon to coyotes and subsequently determining the numbers of trematode eggs/g of feces and numbers of *N. salmincola* recovered at necropsy. Results of the experiment indicated that praziquantel at the dosages and routes of administration used was not effective against metacercariae in chinook salmon.

Key words: *Nanophyetus salmincola*, chinook salmon, *Oncorhynchus tshawytscha*, metacercariae, praziquantel, drug evaluation, experimental infection.

Nanophyetus salmincola is a trematode commonly found in carnivores along coastal Washington, Oregon, and northern California in the northwestern United States (Millemann and Knapp, 1970; Gorham and Foreyt, 1984). The life cycle of the trematode involves a snail (*Oxytrema silicula*) as the first intermediate host and usually a salmonid fish as a second intermediate host. Metacercariae of *N. salmincola* have a predilection for kidney and heart tissue in fish, and when infection is massive, fish often die or are severely debilitated (Baldwin et al., 1967; Wood, 1968; Millemann and Knapp, 1970; Butler and Millemann, 1971), thereby limiting the production of several salmonid species, particularly in hatcheries. Effective control of *N. salmincola* in hatcheries theoretically would reduce mortality in fish and reduce the prevalence of salmon poisoning disease in canines, a life threatening rickettsial disease caused by *Neorickettsia helminthoeca* which is acquired when metacercariae are eaten with infected fish.

In recent experiments, praziquantel was shown to be highly effective against adult *Nanophyetus salmincola* in coyotes (*Canis latrans*) under experimental conditions (Foreyt and Gorham, 1988). The purpose of this experiment was to determine the efficacy of praziquantel against the metacercariae of *N. salmincola* in chinook salmon (*Oncorhynchus tshawytscha*).

Chinook salmon, naturally infected with metacercariae of *N. salmincola* were obtained from McAllister Salmon Hatchery (10119 Steilacoom Road, Olympia, Washington 98503, USA). The fish had a mean weight of 10.9 g, ranging from 5.1 to 14.6 g. The mean number of metacercariae in a random sample of 10 fish was 370; these ranged from 222 to 560 metacercariae/fish. Methods for determining numbers of metacercariae per fish have been reported previously (Foreyt et al., 1987). Salmon were divided randomly into five treatment groups of 10 fish per group. Each fish was treated individually on 20 May 1987. Each fish in group I was given praziquantel powdered tablets (Droncit tablets, Bayvet Division, Miles Laboratories, Shawnee, Kansas 66201, USA) orally in number 5 gelatin capsules with a forceps at a dosage of 10 mg/kg body weight (BW). Fish in group II were given praziquantel powdered tablets orally in gelatin capsules at a dosage of 100 mg/kg BW. Fish in group III were given praziquantel solution (Droncit injectable, Bayvet Division, Miles Laboratories Inc., Shawnee, Kansas 66201, USA) orally in number 5 gelatin capsules at a dosage of 100 mg/kg BW. Fish in group IV were given praziquantel solution