

SYSTEMIC NEOSPOROSIS IN A WHITE RHINOCEROS

Angkana Sommanustweechai, D.V.M., Montakan Vongpakorn, D.V.M., M.Sc., Tanit Kasantikul, D.V.M., Jedsada Taewnean, D.V.M., Boripat Siriaronrat, D.V.M., Ph.D., Mitchell Bush, D.V.M., Dipl. A.C.Z.M., and Nopadon Pirarat, D.V.M., Ph.D.

1

Abstract: Neosporosis was diagnosed in a 16-year-old female white rhinoceros (*Ceratotherium simum*) that died suddenly without clinical signs. Histopathology revealed disseminated protozoan tachyzoites in liver, adrenal cortex, kidney, and intestine, with morphology compatible with either *Toxoplasma* or *Neospora*. The organism was identified as *Neospora caninum* with the use of primary rabbit anti-*N. caninum* antibody immunohistochemistry and polymerase chain reaction. The exact source of infection remains unknown, but it is suspected that *N. caninum* oocysts were ingested from the soil.

Key words: *Ceratotherium simum*, *Neospora caninum*, neosporosis, systemic, white rhinoceros.

BRIEF COMMUNICATION

Captive white rhinoceros (*Ceratotherium simum*) are susceptible to several infections. Neosporosis is a disease caused by *Neospora caninum*, a cyst-forming protozoon of the phylum Apicomplexa, closely related to *Toxoplasma gondii*. *Neospora caninum* was first reported in dogs and later named *N. caninum*.³ Recently, these organisms have been found to be widespread among domestic and several species of wild animals. *Neospora caninum* causes paralysis and death in dogs, as well as neonatal mortality; abortion in cattle,² sheep, goats, and horses;⁵ and death and stillbirth in deer.^{4,11} Vertical transmission is the common route for neosporosis; however, horizontal transmission can also occur from the shedding of oocysts in domestic and wild canids.⁷

A wild-caught 16-yr-old male white rhinoceros was housed at a zoo in Thailand with a female for 15 yr. In the same enclosure were chickens, ostriches, zebras, waterbucks, springboks, wildebeests, and giraffes. Feral cats, dogs, pigeons, deer, and primates were present on the zoo grounds. The rhinoceros were fed freshly cut grass and horse concentrate daily. The affected animal was clinically normal until it was found in sternal recumbency in the morning and died 3 hr

later. Postmortem examination was performed within 2 hr.

On gross pathologic examination, the liver was slightly enlarged with rounded edges and contained multifocal, firm, yellowish necrotic areas (1–13 cm in diameter; Fig. 1A) involving approximately 60% of the liver. The lungs were severely hemorrhagic and edematous. Diffuse hemorrhages were observed in the diaphragm. Hemorrhagic infarction was found in the spleen. The left kidney contained several small necrotic areas (2–5 cm). There was a necrotic area on the mesentery close to the duodenum. The mesenteric lymph nodes and adrenal gland were markedly enlarged and contained diffuse ecchymotic hemorrhages. Gastric erosions and ulcerations were also noted. The color of the jejunal mucosa was black with a large focal hemorrhage 3 cm in diameter. Tissue samples from brain, liver, spleen, lung, kidney, myocardium, and adrenal gland were collected in 10% neutral buffered formalin for histologic examination.

Histopathologically, severe, diffuse, necrotic foci surrounded by plasma cells and lymphocytes were found in the liver, adrenal cortex, kidney, and small intestine. Chronic hepatic fibrosis with atypical granulomatous inflammation was characterized by a central necrotic zone and numerous giant cells and macrophages without surrounding fibrosis. Necrotic lymphadenitis and splenitis was associated with lymphoid depletion and neutrophil infiltration. Numerous tachyzoites were observed in liver, adrenal cortex, and kidneys (Fig. 1B–D). No protozoan cysts were detected in brain or heart. Pulmonary hemorrhages and edema were severe.

Immunohistochemical examination with primary rabbit anti-*N. caninum* antibody (provided by Dr. M. McAllister, Illinois, USA) revealed

From the Zoological Park Organization, 71 Rama 5, Dusit, Bangkok, 10300 Thailand (Sommanustweechai, Kasantikul, Siriaronrat); the Khao Kheow Open Zoo, 237 Moo 7 Bangpra, Sri Racha, Chonburi, 20110 Thailand (Taewnean, Bush); the Parasitology Section, National Institute of Animal Health Kaset Klang, Chatuchak, Bangkok, 10900 Thailand (Vongpakorn); and the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, 10130 Thailand (Pirarat). Correspondence should be directed to Dr. Angkana (angankana@yahoo.com).

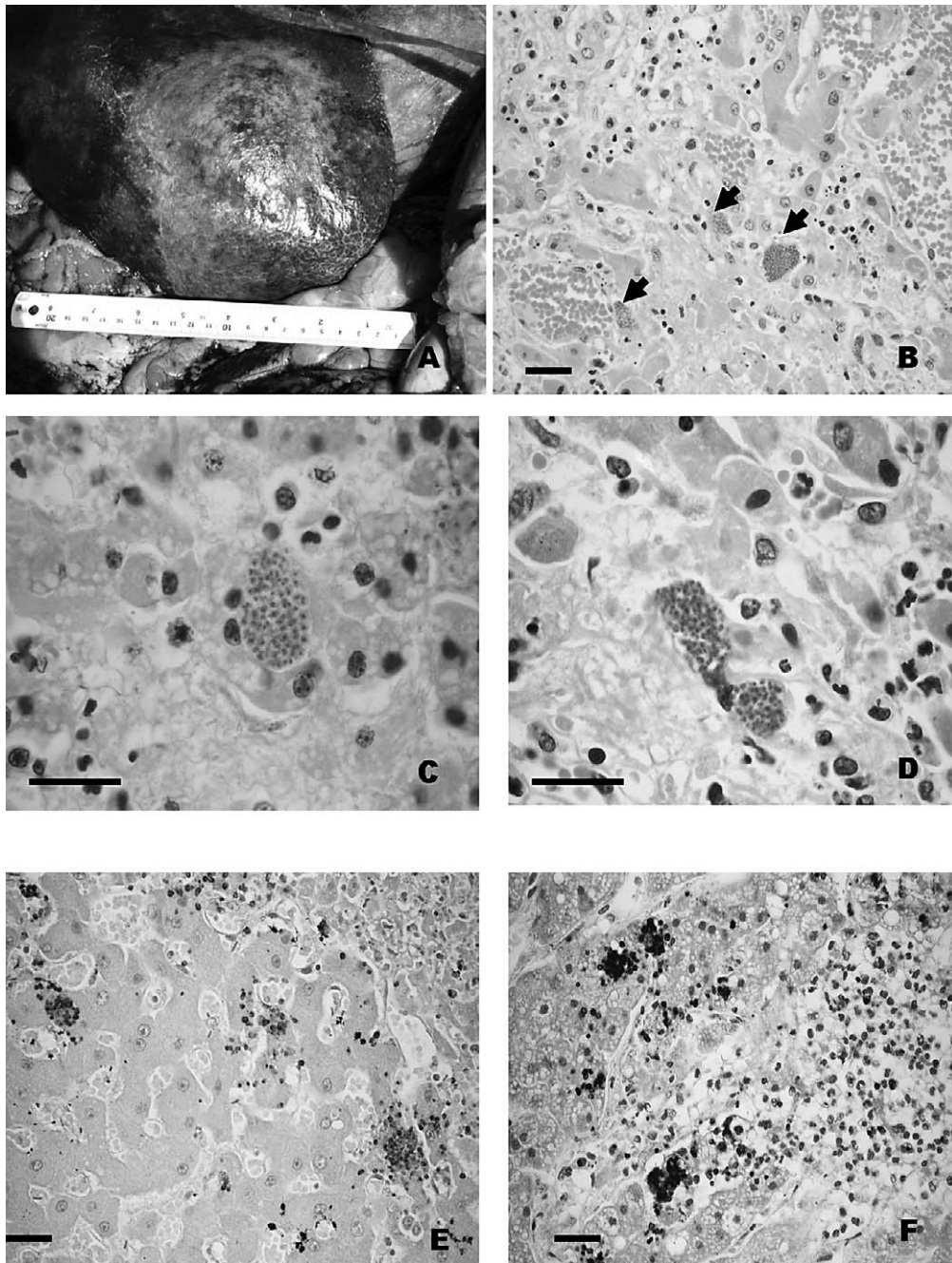


Figure 1. Necropsy revealed the massive necrotic areas. Various sizes from 1 to 13 cm in diameter, were multifocally seen in liver (A). Histopathologically, numerous protozoal structures were detectable in the liver (B, C) and adrenal cortex (D). Positive immunohistochemical signals were obviously observed in the necrotic areas in the liver (E) and the adrenal gland (F). Bar = 25 μm.

positive signals predominantly distributed in the liver (Fig. 1E), adrenal cortex (Fig. 1F), kidney, and small intestine. The *N. caninum* antigen was identified in phagocytic cells, hepatocytes, intes-

tinal and bile duct epithelia, and capillary endothelia. Immunolabeling in the liver was observed in the hepatocytes, sinusoidal endothelium, Kupffer cells, and, occasionally, bile duct

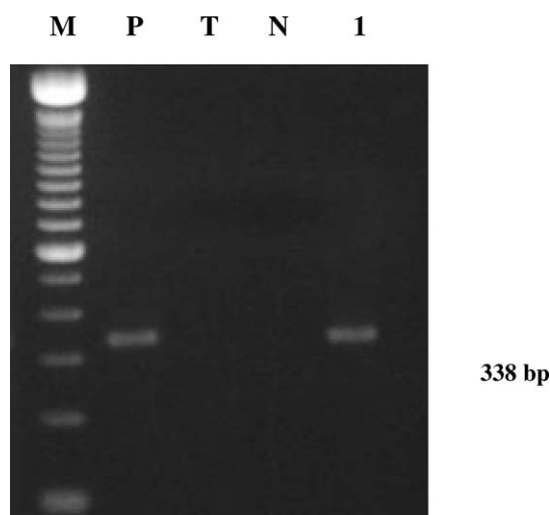


Figure 2. Agarose gel electrophoresis of 328-bp product by *Neospora caninum*-specific primers. Lane M, 100-bp molecular weight marker; P, positive control (*N. caninum* DNA); N, negative control (distilled water); T, *Toxoplasma gondii* DNA; 1, liver.

epithelium. No positive signal was observed in brain and heart.

A polymerase chain reaction (PCR) using primer Np-6 and Np-21 (Primer sequence)¹² with some modifications (PCR procedure) was used in this case. The results showed a specific band at 328 base pairs for *N. caninum* (Fig. 2) in the liver.

In this report, we document a disseminated *N. caninum* in a captive white rhinoceros without obvious symptoms. A report of a stillborn white rhinoceros calf from neosporosis was documented in South Africa.¹⁰ Additionally, abortion is the most common sign in intermediate hosts such as cattle,² whereas paresis and paralysis of hind limbs are common clinical signs in definitive hosts such as dogs younger than 6 mo of age.¹ Neosporosis in the horse, a species related to rhinoceros, has been documented with myeloencephalitis⁹ or severe visceral infection from tachyzoites present in visceral organs such as the mesenteric lymphoid tissue and small intestine.⁸ Gray et al.⁸ also reported changes in liver and spleen without any disseminating tachyzoites.

Histologic examination revealed multifocal necrotic areas in the liver, adrenal cortex, kidney, and small intestine. The induction of necrotic cell death observed in this case might be due to the rapid replication of tachyzoites in various tissues. The unusual granulomatous response around bradyzoites, together with severe lymphoid de-

pletion, suggests an immunologic response from the rupturing of degenerating cysts. The route of transmission in this case was suspected to be by oral transmission. The tachyzoites replicated in the small intestine penetrated the intestinal mucosal layer, entered the blood stream, and spread to other internal organs. The immunohistochemical results revealed tachyzoites of *N. caninum* antigen in the liver, kidney, small intestine, and adrenal cortex. PCR with the Np21/Np6 primer pair of oligonucleotides was able to demonstrate the 328-bp fragments for *N. caninum* in hepatic tissue.

In epidemiologic studies, *N. caninum* has a wide wildlife host range, including deer and other free-ranging ruminants.^{2,4,6,10,11} Wild felids⁶ and canids, or even stray dogs, can serve as either intermediate or definitive hosts and are important routes of transmission for this parasite.⁷ Two of four dogs that ingested *N. caninum* sporulated oocysts from deer brain had oocysts in their feces.⁷ In orally inoculated mice, tissue cysts were found in their brains after 17 days.¹ On the basis of these studies, it appears that free-ranging animals in the vicinity could be a source of contamination. Sporulated oocysts could contaminate soil, water, and food.⁵ However, there is a lack of information regarding the survival of oocysts in environment.

Future studies are needed to understand the epidemiology of this disease and to prevent the spread of *Neospora* from free-living wildlife to captive animals.

Acknowledgments: The authors thank Dr. Daraka Tongthainan, Dr. Piyanee Ngamsuk, and all individuals who assisted in the postmortem examination on the rhinoceros. The authors thank Dr. M. McAllister for supplying primary antibodies; Dr. Sawang Kerdangsakulwut in the Pathology Unit of the Faculty of Veterinary Science, Chulalongkorn University; Ms. Mayuree Waiyakroot in the Parasitology section, National Institute of Animal Health, who assisted in the diagnosis; and Mr. Supradit Wangnaithum for assistance with the laboratory diagnosis. *Neospora* DNA and *Toxoplasma* DNA were kindly provided by Dr. Yaowaluk Suktana, Faculty of Tropical Medicine, Mahidol University.

LITERATURE CITED

1. Dubey, J. P. 1999. Recent advances in *Neospora* and neosporosis. *Vet. Parasitol.* 84: 349–367.
2. Dubey, J. P. 2003. Neosporosis in cattle. *J. Parasitol.* 89: 42–56.

3. Dubey, J. P., J. L. Carpenter, C. A. Speer, M. J. Topper, and A. Uggla. 1988. Newly recognized fatal protozoan disease of dogs. *J. Am. Vet. Med. Assoc.* 192: 1269–1285.
4. Dubey, J. P., J. Rigouler, P. Lagourette, C. George, L. Longeart, and J. L. LeNet. 1996. Fatal transplacental neosporosis in a deer (*Cervus eldi siamensis*). *J. Parasitol.* 82: 338–339.
5. Dubey, J. P., G. Schares, and L. M. Ortega-Mora. 2007. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.* 20: 323–367.
6. Ferroglio, E., E. Wambwa, M. Castiello, A. Trisciuoglio, A. Prouteau, E. Pradere, S. Ndungu, and D. De. Meneghi. 2003. Antibodies to *Neospora caninum* in wild animals from Kenya, East Africa. *Vet. Parasitol.* 118: 43–49.
7. Gondim, L. F. P., M. M. McAllister, N. E. Mateus-Pinilla, W. C. Pitt, L. D. Mech, and M. E. Nelson. 2004. Transmission of *Neospora caninum* between wild and domestic animals. *J. Parasitol.* 90: 1361–1365.
8. Gray, M. L., B. G. Harmon, L. Sales, and J. P. Dubey. 1996. Visceral neosporosis in a 10-year-old horse. *J. Vet. Diag. Invest.* 8: 130–133.
9. Hamir, A. N., S. J. Tornquist, T. C. Gerros, M. J. Topper, and J. P. Dubey. 1998. *Neospora caninum*-associated equine protozoal myeloencephalitis. *Vet. Parasitol.* 79: 269–274.
10. William, J. H., I. Espie, E. van Wilpe, and A. Matthee. 2002. Neosporosis in a white rhinoceros (*Ceratotherium simum*) calf. *J. S. Afr. Vet. Assoc.* 73: 38–43.
11. Woods, L. W., M. L. Anderson, P. K. Swift, and K. W. Sverlow. 1994. Systemic neosporosis in a California black-tailed deer (*Odocoileus hemionus columbianus*). *J. Vet. Diagn. Invest.* 6: 508–510.
12. Yamage, M., O. Flechtner, and B. Gottstein. 1996. *Neospora caninum*: specific oligonucleotide primers for the detection of brain “cyst” DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). *J. Parasitol.* 82: 272–279.

Received for publication 4 July 2009

*Authors Queries*Journal: **Journal of Zoo and Wildlife Medicine**Paper: **zamd-41-01-28**Title: **SYSTEMIC NEOSPOROSIS IN A WHITE RHINOCEROS**

Dear Author

During the preparation of your manuscript for publication, the questions listed below have arisen. Please attend to these matters and return this form with your proof. Many thanks for your assistance

Query Reference	Query	Remarks
1	Author: This article has been lightly edited for grammar, style, and usage. Please compare it with your original document and make corrections on these pages. Please limit your corrections to substantive changes that affect meaning. If no change is required in response to a question, please write "OK as set" in the margin. Copy editor	