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TOXOPLASMOSIS IN NILGAIS (*BOSELAPHUS TRAGOCAMELUS*) AND A SAIGA ANTELOPE (*SAIGA TATARICA*)

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Abstract: Three captive female nilgais (*Boselaphus tragocamelus*) either showed late-term abortion or their newborn calves died within 2 days of birth. *Toxoplasma gondii* DNA was demonstrated in the brain and liver of each fetus and in one of the two neonates by single-stage polymerase chain reaction (PCR) with TGR1E and by seminested PCR with B1 gene. Retrospectively, antibodies titers ≥640 to *T. gondii* by indirect fluorescence were found in the sera of all three female and one male nilgais. No other cause of abortion was detected. Fatal toxoplasmosis was also diagnosed in one captive, adult female saiga antelope (*Saiga tatarica*), which died suddenly. *Toxoplasma gondii* was detected in the liver, lung, spleen, kidney, and intestine. An unusual finding was the presence of numerous tissue cysts in the liver of this animal. Toxoplasmosis was confirmed by PCR with TGR1E and immunohistochemically. Toxoplasmic hepatitis and pneumonia were considered to be the primary causes of death.

Key words: Abortion, *Boselaphus tragocamelus*, bovids, PCR, *Saiga tatarica*, *Toxoplasma gondii*.

INTRODUCTION

The protozoan parasite *Toxoplasma gondii* is prevalent worldwide in domestic and wild mammals and birds.[6] Toxoplasmosis is a major cause of abortion and stillbirth in sheep and goats and causes other disease syndromes in many species of wild and domestic animals. Toxoplasmosis has been described in various exotic bovids in zoologic gardens, including slender-horned gazelle (*Gazella leptoceros*), dama gazelle (*G. dama*), Cuvier's gazelle (*G. cuvieri*), gerenuk (*Litocranius walleri*), dik-dik (*Madoqua guentheri*), and saiga antelope (*Saiga tatarica*).[2,9,11,14,16,17] Disseminated *Toxoplasma*-like protozoan infection was also described in a 2-day-old Lichtenstein's hartebeest (*Sigmoceros lichtensteinii*).[13] Abortion-associated toxoplasmosis has been reported in Greenland muskox (*Ovibos moschatus*).[4] Fatal toxoplasmosis in saiga antelopes has been described previously in two studies, in both cases diagnosed by conventional histologic examination.[2,11]

This article describes an outbreak of toxoplasmosis in captive nilgais (*Boselaphus tragocamelus*), manifested by abortions and perinatal deaths, and a further case of fatal toxoplasmosis in an adult captive saiga.

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CASE REPORTS

Case 1

Three adult female and one adult male nilgais were kept in a 4,700-m^2 grass enclosure in the Zoological Garden in Prague, Czech Republic for 4 yr, on a diet of hay, vegetables, and grain. In August 1999, the three females had five young within a period of 2 days. Two were late-term abortions, two neonates died 1 or 2 days after birth, and one calf survived. No stillbirths or perinatal deaths were observed in other bovids in the zoo that year.

Necropsies and serologic testing of the nilgais were performed at the State Veterinary Institute in Prague, Czech Republic. The fetuses weighed approximately 3 kg, and the neonates weighed approximately 4 kg. Grossly, the organs of both fetuses were partially autolyzed. Livers were enlarged, with small white foci in three of four fetuses and neonates, and two fetuses showed evidence of acute pneumonia. Both fetuses had mandibular prognathism and one also showed hydrocephalus internus. Specimens of liver and lung were fixed in 10% neutral buffered formalin. Paraffin-embedded, 5- to 6-μm sections were stained with hematoxylin and eosin. The hepatic lesions consisted of focal hemorrhagic necrosis. The pulmonary lesions consisted of acute interstitial pneumonia. Routine bacteriologic culture of parenchymatous organs and stomach contents were negative for known ruminant pathogens.

Table 1. Reciprocal antibody titers to *Toxoplasma gondii* in adult nilgais detected by indirect fluorescence.

Nilgai no.	Sex	3 mo before abortion	5 wk after abortion	9 mo after abortion
1	Male	5,120	ND	640
2	Female	ND[a]	640	640
3	Female	ND	ND	1,280
4	Female	ND	ND	640

[a] ND = not done.

Serum collected from the adult male nilgai 3 mo before the abortion, from one female 5 wk after the abortion, and from all four dams 9 mo after parturition was tested for a variety of pathogens. Antibody titers for bovine herpesvirus-1, bovine viral diarrhea virus, *Brucella abortus*, *Coxiella burnetti*, *Chlamydophila abortus*, *Listeria monocytogenes*, *Neospora caninum*, and 10 leptospiral serotypes were negative. Antibodies to *T. gondii* were tested by a latex agglutination test (LAT) and indirect fluorescence antibody test (IFAT). The LAT (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) was performed according to the manufacturer's instructions. Sera were diluted 1:10 and higher. For IFAT, a commercially available antigen (Sevatest Toxoplasma Antigen IFR, Sevac, Prague, Czech Republic) diluted according to the manufacturer's instructions and swine anti-bovine immunoglobulin SwAB/FITC (Sevac) at a 1:20 dilution were used. Sera were diluted in a twofold series starting at 1:20. Titers ≥1:20 were considered positive. Serum samples obtained from a naturally infected yak (*Bos mutus*) and uninfected domestic cattle were used as positive and negative controls, respectively. Antibodies to *T. gondii* were detected in all serum samples by LAT and IFAT. The IFAT titers are shown in Table 1.

Polymerase chain reaction (PCR) was used to detect *T. gondii* DNA in the following tissues: brain, liver, lung, kidney, spleen, and heart of the four fetuses and neonates. Two parallel samples of DNA from each tissue were isolated. Single-stage PCR with the specific primers of repetitive sequence TGR1E was used.[5] The conditions of amplification were 94°C 3 min, 35 cycles: 94°C 30 sec, 60°C 30 sec, 72°C 30 sec, 72°C 7 min. Product of PCR (191 bp) was analyzed on 2% agarose gel with ethidium bromide. Beta-globin gene amplification was used as internal control. Seminested PCR was performed with the specific primers of B1 gene[3] under the following conditions: 95°C 3 min, 30 cycles (94°C 30 sec, 65°C 30 sec, 72°C 1 min), 72°C 10 min, 30 cycles (94°C 30 sec, 55°C 30 sec, 72°C 1 min), 72°C 10 min. Specific B1 gene sequence (362 bp) was detected on 2% agarose gel with ethidium bromide. *Toxoplasma gondii* DNA was demonstrated in the brain and liver of the two aborted fetuses and in the brain of one of the two neonates that died perinatally, but not in other tissues (Table 2).

Table 2. Presence of *Toxoplasma gondii* in the tissues of nilgai fetuses and newborn calves as determined by polymerase chain reaction using TGR1E and B1 sequences.

	Organs	TGR1E	B1	Internal control
Fetus 1	brain	+	+	+
	liver	+	+	+
	lung	−	−	+
Fetus 2	brain	+	+	+
	liver	−	+	+
	lung	−	−	+
	kidney	−	−	+
	spleen	−	−	+
	heart	−	−	+
Neonate 1	brain	−	−	+
	liver	−	−	+
	lung	−	−	+
	kidney	−	−	+
	spleen	−	−	+
	heart	−	−	+
Neonate 2	brain	+	+	+
	liver	−	−	+
	lung	−	−	+
	kidney	−	−	+
	spleen	−	−	inhibition
	heart	−	−	+

Case 2

A group of saiga antelope from the Askania Nova Biospheric Reserve, Ukraine, were imported into the Czech Republic in 1997, 1999, and 2000. The herd was kept in a 6-ha grass enclosure in the Podkrušnohorský Zoo Park, Chomutov, Czech Republic. They were fed hay, vegetables, and grain and were also pastured. Two male saiga, 5- and 6-mo-old, died in October and November 2001, respectively. One animal was infected with *Muellerius* sp., *Nematodirus* sp., and *Trichocephalus* sp. The second animal was not examined. In December 2001, three adult (two females and one male) and one young male saiga died. A 3-yr-old female (no. 1) and a 7-mo-old male died on 16 December. Examination was limited to the lungs for the presence of lungworm. Massive *Muellerius* sp. infection was found in the female, and mild infestation was found in the male. A 1.5-yr-old female (no. 2) died on 19 December, and a 1.5-yr-old male died on 27 December, ostensibly because of head trauma. All an-

imals died suddenly without apparent illness. In that same year, no cases of death or abortion occurred in other bovids in the Zoo Park.

Liver, lung, spleen, kidney, and intestinal tissues from female no. 2 were submitted within 48 hr of death to the State Veterinary Institute, Prague. The lungs were edematous and hemorrhagic. Widespread splenic necrosis and mild hepatomegaly were also present. All organs were screened by routine bacteriologic culture, and the lungs were examined for the presence of lungworms. *Pasteurella multocida* was isolated from the lungs, and mild *Muellerius* sp. infection was present. Impression smears of the liver were stained using Diph Quick (Medical Product, Pardubice, Czech Republic) and examined microscopically. Pieces of liver were fixed in 10% neutral buffered formalin, and paraffin-embedded 5- to 6-μm sections were stained with hematoxylin and eosin, and periodic acid Schiff (PAS). Paraffin blocks of the liver were sent to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for immunohistochemical examination using *T. gondii* and *N. caninum* rabbit antibodies by a method described previously.[7] The *T. gondii* polyclonal antibodies used stained all stages of *T. gondii*. In addition, a bradyzoite-specific BAG-1 antibody was used.[7] Several tissue cysts were seen in impression smears of liver. Hepatic lesions consisted of focal necrosis, perivascular infiltrates, and hemorrhage. Tissue cysts and tachyzoites were seen in hepatocytes histologically. Organisms in the liver reacted with polyclonal *T. gondii* antibodies but not with antibodies to *N. caninum*. The organisms also reacted with BAG-1 antibodies, indicating they were bradyzoites. Tissue cysts in the liver were PAS positive.

Frozen specimens of liver, lungs, kidneys, spleen, and intestine were submitted to the Department of Biology and Wildlife Disease, University of Veterinary and Pharmaceutical Science, Brno, Czech Republic. Single-stage PCR with the specific primers of repetitive sequence TGR1E, as described above, was used to detect *T. gondii* DNA. *Toxoplasma gondii* DNA was found in all tissues examined.

Serum samples collected from 10 healthy saiga antelope during 1999–2000 were tested retrospectively for *T. gondii* antibodies by IFAT and LAT by the same procedures described above. All were seronegative.

DISCUSSION

Nilgais are robust Asian antelopes commonly kept in zoologic gardens. The abortions and perinatal deaths in the nilgais were caused by toxoplasmosis, based on the presence of *T. gondii* DNA in tissues and the absence of other significant findings. Our results were similar to those using single-tube, nested PCR from sheep fetuses, where *T. gondii* was also detected predominantly in brain.[10] The prevalence of antibodies to *T. gondii* in adult nilgai was 100% using both LAT and IFAT. The highest IFAT titer using heterologous anti-bovine conjugate was 5,120. It is not known whether the malformations in the aborted fetuses were associated with toxoplasmosis.

Latent *T. gondii* infection without clinical toxoplasmosis has been identified serologically in nilgais kept in German and Polish zoos.[12] The only exotic bovid reported with toxoplasmosis-associated abortion was a Greenland muskox (*Ovibos moschatus*) kept in the San Francisco Zoo.[4] In the muskox, tachyzoites of *T. gondii* were identified by immunohistochemistry on the placenta. Antibodies against *T. gondii* were measured by modified agglutination test at the time of abortion, as well as during the previous 3 yr, with titers ≥3,200. The only ruminants (other than the family Bovidae) reported with toxoplasmic abortion are reindeer (*Rangifer tarandus*).[8]

To date, there have been few reports of infectious disease in captive nilgais and no cases of abortion caused by infectious agents. Recently, neosporosis associated with stillbirth was reported in lesser kudu (*Tragelaphus imberbis*),[15] a related species. Nilgai, lesser kudu, and domestic cattle belong to the subfamily Bovinae. Toxoplasmic abortion or fatal toxoplasmosis in this subfamily has not been reported, whereas it is common in goats and sheep of the subfamily Caprinae.[6]

Saiga antelopes are unusual, medium sized, endangered, and live on the dry steppes and semideserts of central Asia. They are rarely kept in European zoologic gardens. Fatal toxoplasmosis was first reported in saiga in 1971, in three animals,[2] and again in 10 saiga (1- to 3.5-yr-old) that died between 1961 and 1979 in the Berlin Zoo.[11] The majority of affected animals showed necrosis and cellular infiltrates in the liver, catarrhal and necrotizing pneumonia, gastroenteritis, lymphadenitis, and nephritis. Hepatic necrosis with granulomas, endometritis, fibrinous epicarditis, myositis, and fibrinous peritonitis were seen sporadically. *Toxoplasma* tissue cyst–like structures were found in the livers of all animals, in most lungs, and intestines but only occasionally in kidneys, skeletal muscles, and endometrium.

Our finding of *T. gondii* cysts in the liver corresponds with previous descriptions of the disease in Cuvier's gazelles.[14] Because *T. gondii* was dem-

onstrated in all tissues examined, we considered disseminated toxoplasmosis to be the cause of death of the saiga, despite the presence of *P. multocida* and *Muellerius* sp. in the lung. It is not known whether infection with these organisms influenced the pathogenesis of toxoplasmosis. Recently, concurrent toxoplasmic encephalitis and pneumonic pasteurellosis was described in a free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis*) from Washington.[1] It was not determined whether the death of these sheep was the result of severe *Pasteurella* bronchopneumonia or *Toxoplasma* encephalitis.[1]

We suspect that infection was most likely derived from *T. gondii* oocysts spread from feces of infected cats. Domestic cats commonly live and raise kittens in the runs, houses, and haylofts of hoofed mammals in both zoos. It appears that saiga antelope are highly susceptible to *T. gondii* infection and usually develop the lethal form of toxoplasmosis.

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LITERATURE CITED

1. Baszler, T. V., J. P. Dubey, C. V. Löhr, and W. J. Foreyt. 2000. Toxoplasmic encephalitis in a free-ranging rocky mountain bighorn sheep from Washington. J. Wildl. Dis. 36: 752–754.

2. Bulmer, W. S. 1971. Toxoplasmosis in captive saiga antelope. J. Wildl. Dis. 7: 310–317.

3. Burg, J. L., C. M. Grover, P. Pouletty, and J. C. Boothroyd. 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbiol. 27: 1787–1792.

4. Crawford, G. C., F. H. Dunker, and J. P. Dubey. 2000. Toxoplasmosis as a suspected cause of abortion in a Greenland muskox (*Ovibos moschatus wardi*). J. Zoo Wildl. Med. 31: 247–250.

5. Cristina, N., B. Oury, P. Ambroise-Thomas, and F. Santoro. 1991. Restriction-fragment-length polymorphisms among *Toxoplasma gondii* strains. Parasitol. Res. 77: 266–268.

6. Dubey, J. P., and C. P. Beattie. 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida.

7. Dubey, J. P., M. W. Garner, M. M. Willette, K. L. Batey, and C. H. Gardiner. 2001. Disseminated toxoplasmosis in magpie geese (*Anseranas semipalmata*) with large numbers of tissue cysts in livers. J. Parasitol. 87: 219–223.

8. Dubey, J. P., B. Lewis, K. Beam, and B. Abbit. 2002. Transplacental toxoplasmosis in a reindeer (*Rangifer tarandus*) fetus. Vet. Parasitol. 110: 131–135.

9. Dubey, J. P., M. E. Tocidlowski, B. Abbitt, and S. Y. Llizo. 2002. Acute visceral toxoplasmosis in captive dik-dik (*Madoqua guentheri smithi*). J. Parasitol. 88: 638–641.

10. Hurtado, A., G. Aduriz, B. Moreno, J. Barandika, and L. García-Pérez. 2001. Single tube nested PCR for detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. Vet. Parasitol. 102: 17–27.

11. Ippen, R., J. Jíra, and K. Blaek. 1981. Toxoplasmose als Todesursache bei Saiga-Antilopen (*Saiga tatarica*). Proc. Int. Symp. über die Erkrankungen der Zootiere 23: 185–191.

12. Ippen, R., V. Kozojed, and J. Jíra. 1981. Toxoplasmosis in zoo animals. Folia Parasitol. 28: 109–115.

13. Jardine, J. E., and J. P. Dubey. 1996. Systemic infection with an unidentified *Toxoplasma*-like protozoan in a neonatal Lichtenstein hartebeest (*Sigmoceros lichtensteinii*). J. Parasitol. 82: 515–517.

14. Junge, E. R., J. R. Fischer, and J. P. Dubey. 1992. Fatal disseminated toxoplasmosis in a captive Cuvier's gazelle (*Gazella cuvieri*). J. Zoo Wildl. Med. 23: 342–345.

15. Peters, M., P. Wohlsein, A. Knieriem, and G. Scharres. 2001. *Neospora caninum* infection associated with stillbirths in captive antelopes (*Tragelaphus imberbis*). Vet. Parasitol. 97: 153–157.

16. Stiglmair-Herb, M. T. 1987. Mikroparasitose (Toxoplasmose) bei Edmigazellen (*Gazella g. cuvieri*). Berl. Muench. Tieraerztl. Wochenschr. 100: 273.

17. Stover, J., E. R. Jacobson, J. Lukas, M. R. Lappin, and C. D. Buergelt. 1990. *Toxoplasma gondii* in a collection of nondomestic ruminants. J. Zoo Wildl. Anim. Med. 21: 295–301.

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