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Authors: Fayer, Ronald, Fischer, John R., Sewell, Christopher T., Kavanaugh, Darrell M., and Osborn, David A.

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## SPONTANEOUS CRYPTOSPORIDIOSIS IN CAPTIVE WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

Ronald Fayer,<sup>1</sup> John R. Fischer,<sup>2</sup> Christopher T. Sewell,<sup>2</sup> Darrell M. Kavanaugh,<sup>2</sup> and David A. Osborn<sup>3</sup>

<sup>1</sup> United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, Maryland 20705, USA

<sup>2</sup> Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA

<sup>3</sup> The Warnell School of Forest Resources, The University of Georgia, Athens, Georgia, 30602 USA

**ABSTRACT:** In August 1994, cryptosporidiosis was diagnosed in a diarrheic fawn from a captive white-tailed deer (*Odocoileus virginianus*) herd maintained for research purposes at The University of Georgia's Warnell School of Forest Resources in Athens, Georgia (USA). From June through August 1995, 11 captive female white-tailed deer were housed in individual barn stalls where they gave birth to 18 fawns. Feces collected at 2 or 3 day intervals from the 18 neonatal fawns for at least 21 days and from 11 adult females once from 1 to 30 days before fawns were born and on three to 12 occasions after their birth were examined for oocysts of *Cryptosporidium* spp. Feces from all animals appeared normal throughout the period of examination. Oocysts morphologically indistinguishable from those of *Cryptosporidium parvum* were detected intermittently in the feces of one adult female from 1 to 25 days after parturition and in the feces of her fawn from 11 to 22 days of age. Oocysts also were detected intermittently in feces from twin fawns from 9 to 20 days of age, but not from their mother. Oocysts from deer were infectious for neonatal mice as determined histologically, and for calves as determined by clinical signs and excretion of oocysts.

**Key words:** Cryptosporidiosis, *Cryptosporidium parvum*, white-tailed deer, *Odocoileus virginianus*, captive deer.

### INTRODUCTION

Oocysts indistinguishable from those of *Cryptosporidium parvum* have been identified in feces of 79 species of mammals including human beings (O'Donoghue, 1995). In Scotland and New Zealand, epizootics of cryptosporidiosis in captive red deer (*Cervus elaphas*) calves were characterized by diarrhea and high mortality (Tzipori et al., 1981; Orr et al., 1985). Cryptosporidiosis has been reported in captive deer such as roe deer (*Capreolus capreolus*) in Denmark (Korsholm and Henriksen, 1984), and in fallow deer (*Cervus dama*), sika deer (*Cervus nippon*), mule deer (*Odocoileus hemionus*), Eld's deer (*Cervus eldi thamin*), axis deer (*Cervus axis*), and barasingha deer (*Cervus duvauceli*) at the San Diego Wild Animal Park (Heuschele et al., 1986). Virtually all deer were neonates with diarrhea. Although they are the most prevalent wild ruminants in North America, there have been no published reports of *Cryptospor-*

*idium* spp. in wild or captive white-tailed deer (*Odocoileus virginianus*).

In August 1994, cryptosporidiosis was diagnosed in a white-tailed deer fawn with diarrhea that was part of a captive herd at The University of Georgia's Warnell School of Forest Resources in Athens, Georgia (USA) (J. R. Fischer, unpubl.). Our objectives were to determine if captive adult or neonatal white-tailed deer were naturally infected with *Cryptosporidium* spp. at that facility, to monitor clinical signs and fecal excretion of oocysts in infected deer, and to identify protozoal oocysts by their morphology and ability to infect mice and calves.

### MATERIALS AND METHODS

From 1 June through 25 August 1995, 11 pregnant female white-tailed deer (*Odocoileus virginianus*) were individually confined within 3 × 6 m barn stalls. Stalls had 1.5-m high wooden walls and welded wire fencing extended from the tops of the walls to the open trusses of the roof. Stall floors were clay and were covered with approximately 5 cm of clean, dry,

wood shavings that were replaced every 7 to 10 days. Deer had an 18% protein pelleted ration (Gold Kist Inc., Atlanta, Georgia) and fresh water available ad libitum; diets were supplemented with alfalfa (*Medicago sativa*) hay.

The adult females in this study were from a variety of sources and included animals that had been born and reared at the facility, orphaned wild animals submitted to the facility for fostering by resident females, and one adult deer that had been removed from a cattle herd. The deer at this facility had been kept in various outdoor paddocks and indoor stalls prior to initiation of this study. The facility had housed fallow deer and domestic sheep during research projects in previous years.

Feces were collected from adult females once from 1 to 30 days before fawns were born, and on three to 12 occasions at intervals  $\leq 3$  days beginning when fawns were born, and extending for 1 to 3 wk. Feces were collected from fawns on eight to 12 occasions at intervals  $\leq 3$  days from on or near the day of birth until 3 to 4 wk of age. Feces were collected directly from the rectum of each animal and sealed in plastic bags which were labelled and stored at 5 C until examined.

Feces were first examined at the Southeastern Cooperative Wildlife Disease Study, The University of Georgia, Athens, Georgia. Approximately 1 g of feces was suspended in 15 ml of Sheather's sugar solution (Levine, 1973) and centrifuged  $1,200 \times G$  for 30 min. A glass coverslip was pressed against the supernatant surface, placed on a glass slide, and examined by phase-contrast microscopy. Feces without preservative were then shipped to the United States Department of Agriculture laboratory in Beltsville, Maryland (USA) where 3 to 5 g from each sample were suspended in 15 ml deionized water (dH<sub>2</sub>O), mixed with 35 ml of Sheather's sugar solution and centrifuged at  $1,200 \times G$  for 30 min. The supernatant surface was aspirated and duplicate specimens were examined by light, phase-contrast and interference-contrast microscopy for oocysts. For all specimens with particles resembling oocysts, the remainder of the sample was screened through a graded series of sieves down to a pore size of 45  $\mu\text{m}$  to remove debris. Sieved feces were suspended in dH<sub>2</sub>O and were placed in 50 ml tubes centrifuged at  $1,200 \times G$  for 30 min, the supernatant discarded, and pellets resuspended in dH<sub>2</sub>O. This process was repeated three times to concentrate oocysts and remove debris. To further clean and concentrate oocysts, pellets were cleaned in a cesium chloride gradient (Kilani and Sekla, 1987). Cesium chloride-cleaned specimens were examined by light, phase-contrast, and interfer-

ence-contrast microscopy. Fifty cleaned oocysts from one fawn were measured by optical micrometer.

Cleaned oocysts from this fawn were stored in aqueous 2.5% potassium dichromate at 5 C and less than 1 mo later were used as inoculum to test infectivity for other mammalian hosts. Five 7-day-old BALB/c mice in one litter each received 10,000 oocysts in 50  $\mu\text{l}$  of dH<sub>2</sub>O by gastric intubation via a 26 gauge gavage needle. One litter of five BALB/c neonates served as uninoculated controls. All mice were euthanized by cervical dislocation and necropsied 96 hr after inoculation. A 2 to 3 mm segment of distal ileum from each mouse was fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5  $\mu\text{m}$ , stained with hematoxylin and eosin, and examined by light microscopy for developmental stages of *Cryptosporidium* spp.

Three male Holstein calves were removed from their mothers at birth before suckling and were taken immediately to a clean, sealed, concrete building, where they were housed individually in 2  $\times$  4 m pens. The interior of this building was painted with epoxy paint and each pen was washed with a lye solution, then detergent, and then rinsed with high pressure steam and allowed to thoroughly dry before calves entered. The cement pen floors were covered with approximately 5 cm of clean sawdust which was replaced every 2 days. Within 24 hr of birth calves were fed colostrum via a nipples bottle and within 48 hr of birth one calf was fed an aqueous suspension of  $1 \times 10^6$  oocysts from a deer fawn. The other calves served as controls. Feces were collected daily directly from calves and examined for oocysts as described.

## RESULTS

The 11 adult female deer gave birth to 18 fawns and one female fostered a neonatal orphan fawn in addition to her single fawn. None of the fawns or their mothers had signs of illness, and all feces appeared normal and nondiarrheic throughout the study.

Oocysts were detected in sucrose floats of feces from three fawns, two of which were twins in the same pen. Oocysts were first detected at 9 and 11 days of age and both fawns excreted low numbers of oocysts (less than 10 per coverslip) until day 20. Oocysts were not detected in feces from their mother with whom they shared

a pen. The third positive fawn excreted oocysts intermittently from 11 through 22 days of age with  $>10^6$  oocysts recovered from some daily specimens. Its mother excreted low numbers of oocysts intermittently for 25 days beginning the day after the fawn was born.

Oocysts from the third fawn ranged from  $3.72$  to  $5.58 \times 4.65$  to  $5.58 \mu\text{m}$  with a mean of  $4.67 \times 5.06 \mu\text{m}$  ( $n = 50$ ). They were morphologically indistinguishable from oocysts of *C. parvum*.

All mice that were inoculated with oocysts from deer had developmental stages of *Cryptosporidium* spp. in ileal epithelium. *Cryptosporidium* spp. was not detected in ileum from any of the uninoculated controls.

The calf fed fawn-derived oocysts developed diarrhea and excreted oocysts from 3 to 11 days post inoculation. Virtually all feces were collected from the calf during this time. Over  $5 \times 10^8$  oocysts were recovered from this calf. Both uninoculated controls began excreting oocysts 4 days after the inoculated calf stopped excreting oocysts. Control calves excreted oocysts for 5 to 7 days and both had diarrhea for 6 days.

#### DISCUSSION

This is the first report of infection of white-tailed deer with any species of *Cryptosporidium*. Three of 19 neonatal fawns and one of 11 periparturient females spontaneously excreted oocysts that were morphologically indistinguishable from those of *Cryptosporidium parvum*. Oocysts from these deer readily infected neonatal mice and Holstein calves; this is consistent with experimental cross transmission studies with *C. parvum* (Fayer et al., 1990) and further supports the conclusion that these deer were naturally infected with *C. parvum*. Oocysts found in the feces of the neonatal calf 3 to 11 days after it received oocysts from a white-tailed deer fawn were the most likely source of contamination leading to infection of two control calves housed in adjacent pens in

the same building. An animal caretaker had inadvertently fed and cleaned the pen of the infected calf before entering the pens of the control calves on the last day oocysts were excreted by the inoculated calf. The fact that neither of the control calves excreted oocysts or had diarrhea for 10 days before this event and that both began to excrete oocysts on the same day strongly suggests that the source of their infection was the experimentally infected calf.

The source of infection for these deer was unknown. The most likely source was contamination of the environment in which deer were kept, including barn stalls and outside paddocks. Cryptosporidial oocysts are resistant to many disinfectants and environmental extremes and can remain viable for several months (Fayer et al., 1990). Although oocysts were not detected in feces of female deer prior to parturition, oocyst excretion by a subclinically infected female deer could be a source of infection for fawns. A periparturient rise in oocyst shedding by female sheep was regarded as an initiating factor of cryptosporidiosis in lambs (Xiao et al., 1994). Finally, other animals could have contributed to oocyst contamination of the environment. Oocysts derived from wild mice (*Mus musculus*) were infective for calves and laboratory mice (Klesius et al., 1986).

Based on reports of cryptosporidiosis in many ruminant species, it was not surprising that white-tailed deer also served as hosts of *C. parvum*. The infections in the deer of this study wherein no clinical signs were observed differed from *Cryptosporidium* spp. infections in numerous ruminants including several species of captive deer where diarrhea and death were reported (Tzipori et al., 1981; Korsholm and Henriksen, 1984; Orr et al., 1985; Van Winkle, 1985; Heuschele et al., 1986). However, these reports concern investigations of epizootics of diarrhea in neonatal captive deer, and surveys of healthy deer have not been reported. Although isolates or strains of *C. parvum* might dif-

fer in virulence, infectivity, or other characteristics, a calf inoculated with  $1 \times 10^6$  fawn-derived oocysts was readily infected and had moderate diarrhea. However, the number of oocysts in the infective dose for deer in the present study was unknown. The response of white-tailed deer to infection with known quantities of *C. parvum* oocysts must await future testing.

The role of free-ranging white-tailed deer in disseminating oocysts in the environment is unknown and cannot be extrapolated from the study of captive deer in an artificial setting. Based on present findings, white-tailed deer can be infected with *C. parvum* and excrete oocysts that are infectious to other mammals. Future investigations to determine the prevalence of *C. parvum* in free-ranging white-tailed deer and their role in contributing oocysts to the environment should be focused on surveys of free-ranging deer.

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