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CRYPTOSPORIDIAL INFECTIONS IN CAPTIVE WILD ANIMALS

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ABSTRACT: Neonatal diarrhea was an important cause of morbidity and mortality in a hand-rearing facility for exotic ruminants at the San Diego Wild Animal Park. Studies undertaken to determine the causes of the problem revealed that oocysts of *Cryptosporidium* sp. were demonstrable in auramine O stained fecal smears from 52 of 183 (28.4%) animals examined. Cryptosporidial infection was identified in 21 of 40 species of exotic ruminants with diarrhea. In addition, cryptosporidia were associated with gastroenteric disease in two primates and two reptiles. It was observed also that auramine O stained coccidial oocysts of the genus *Eimeria*, which were present in five of 183 (2.7%) of the specimens examined.

INTRODUCTION

Cryptosporidia are coccidian parasites of the genus *Cryptosporidium* which have been shown recently to be important as causes of gastroenteritis and diarrhea in many species of animals, including humans, domestic and exotic mammals, birds, reptiles and fish (Brownstein et al., 1977; Tzipori and Campbell, 1981; Angus, 1983; Fenwick, 1983; Ma and Soave, 1983; Anderson, 1984; Berk et al., 1984; Cohen et al., 1984; Current, 1984, 1985; Kuller et al., 1984; Navin and Juranek, 1984; Szabo and Moore, 1984; Van Winkle, 1985). The intracellular stages of *Cryptosporidium* sp. are within a vacuole in the microvillous portion of the host gastrointestinal epithelial cells (Current, 1985). Pathologic alterations associated with cryptosporidiosis include villous atrophy, decreased activity of mucosal enzymes, dilatation of intestinal crypts, inflammatory cell infiltration of the lamina propria and diarrhea (Van Winkle, 1985). In infected reptiles, regurgitation is seen more commonly than diarrhea.

An increasing problem of neonatal diarrhea in young hand-raised exotic artiodactyls at the San Diego Wild Animal Park was reported previously by Van Winkle (1985). This paper reports the results of continued studies on the prevalence of *Cryptosporidium* sp. in zoo animals and extends the species range of documented infections in artiodactyls, primates and reptiles.

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MATERIALS AND METHODS

Several methods described for the detection of oocysts of *Cryptosporidium* spp. in feces were evaluated before settling on a single procedure. These included Kinyoun's modification of the Ziehl-Neelsen acid-fast stain (Henriksen and Pohlenz, 1981), iodine solution, Giemsa, Sheather's coverslip flotation (Ma and Soave, 1983), safranin-methylene blue (Baxby et al., 1984), and auramine O (Payne et al., 1983; Heuschele et al., 1984). The use of auramine O fluorochrome dye (Product No. AX1800, Matheson, Coleman and Bell Manufacturing Chemists, Norwood, Ohio 45212, USA) to stain fecal smears was compared preliminarily with the above methods. It proved to be the simplest, most rapid and sensitive method for detecting cryptosporidial oocysts in fecal smears, and was used on all samples in this study. The procedure, as reported previously by Payne et al. (1983) and Heuschele et al. (1984) was as follows:

Stain preparation

1) Dissolve 0.1 g auramine O in 10 ml of 95% ethanol.
2) Mix 3 ml liquefied phenol with 87 ml distilled water.
3) Combine and mix auramine O solution with the phenol-water.
4) Store in amber bottles. Filtering stain is not necessary.

Staining procedure

1) Prepare fecal smears on glass slides and air dry.
2) Flood slides with auramine O stain. Stain for 15 min at room temperature. *Do not heat slides.*
3) Rinse slides with tap water.
4) Decolorize for 2 min with acid-alcohol (0.5% HCl in 70% ethanol).
5) Rinse slides with tap water.
6) Counterstain 3 min with 0.5% aqueous potassium permanganate.
7) Rinse slides with tap water.
8) Allow slides to air dry and examine with fluorescence microscope with BG-12 blue light filter.
9) *Cryptosporidium* oocysts are round to slightly ovoid, 4–7 μm in size, and fluoresce brightly with yellow-gold to yellow-green color. Other coccidial oocysts (e.g., *Eimeria* spp.) were observed to be stained by auramine O, but yeast cells, some of which are similar in size and shape to *Cryptosporidium* sp., did not. *Eimeria* oocysts can be distinguished on the basis of their ovoid morphology and size (13–31 $\times$ 9–25 μm).

The above method was used on fecal specimens from mammals with clinical diarrhea, and in the case of reptiles with regurgitation on gastric fluids and mucosal scrapings.

RESULTS

The prevalence of cryptosporidial infections in neonatal ruminants with diarrhea is summarized in Table 1. A total of 52 of 183 (28.4%) diarrheic neonatal ruminants examined had oocysts of *Cryptosporidium* sp. in fecal smears.

Oocysts of *Cryptosporidium* sp. were found in feces of two of 86 primates with diarrhea: a red-ruffed lemur (*Varecia variegata rubra*) and a cotton-topped tamarin (*Saguinus oedipus*); and two reptiles with clinical signs of gastritis (i.e., regurgitation): a timber rattlesnake (*Crotalus horridus horridus*) and a star tortoise (*Geochelone elegans*).

Auramine O stained oocysts of *Eimeria* sp. were observed in fecal smears from a slender-horned gazelle (*Gazella leptoceros*), a blackbuck (*Antilope cervicapra*), a Persian gazelle (*Gazella subgutturosa*), a Roosevelt's gazelle (*Gazella granti roosevelti*) and an eland (*Taurotragus oryx*).

Cryptosporidium sp. and *Eimeria* sp. were seen easily, glowing with a bright yellow-green fluorescence against an almost black background when auramine O stained smears were examined with a fluorescence microscope.

DISCUSSION

In a previous report by Van Winkle (1985) of morbidity and mortality in neonatal artiodactyls (ruminants) due to diarrhea at the San Diego Wild Animal Park, cryptosporidial infections were detected by histopathologic examination of intestinal sections from animals which had died. He found infections of *Cryptosporidium* sp. in 10 blackbuck, two scimitar-horned oryx, two fringe-eared oryx, and one sable antelope. In addition, he reported the isolation of *Salmonella typhimurium* from 10 of 51 animals examined. Cryptosporidiosis also was reported recently in a Persian gazelle from the Sacramento, California, Zoo (Fenwick, 1983).

The present report expands the list of species susceptible to infection by *Cryptosporidium* sp. Twenty-one of 40 ruminant species, two of three reptile species and two of 14 primate species were infected.

Most recent discussions of cryptosporidiosis have emphasized the fact that the infection is most serious in immunodeficient people or animals (Ma and Soave, 1983; Payne et al., 1983; Berk et al., 1984; Cohen et al., 1984; Current, 1984; Navin and Juranek, 1984). However, lactogenic or colostral immunity is not believed to provide protection against cryptosporidial infection.

Since there is no chemotherapeutic agent reported to be effective in the treatment of cryptosporidiosis or viral causes of diarrhea, therapy of affected animals was mainly supportive, consisting of fluid

TABLE 1. Prevalence of cryptosporidial oocysts in hand-raised neonatal exotic ruminants with diarrhea at the San Diego Wild Animal Park.

Species (common name)	No. positive/ no. examined	% Positive
Aepyceros melampus (impala)	8/9	88.9
Antidorcas marsupialis (springbok)	1/14	7.1
Antilope cervicapra (blackbuck)	4/24	16.7
Bison bonasus (wisent)	0/2	0.0
Bos gaurus (Indian gaur)	0/1	0.0
Boselaphus tragocamelus (nilgai)	5/20	25.0
Capra falconeri (Turkomen markhor)	3/4	75.0
Capra ibex nubiana (Nubian ibex)	0/1	0.0
Connochaetes taurinus albojubatus (white-bearded gnu)	0/1	0.0
Gazella dama ruficollis (Addra gazelle)	1/8	12.5
Gazelle granti roosevelti (Roosevelt's gazelle)	0/2	0.0
Gazella leptoceros (slender-horned gazelle)	4/6	66.7
Gazella subgutturosa (Persian gazelle)	1/10	10.0
Hippotragus equinus (roan antelope)	0/1	0.0
Hippotragus niger (sable antelope)	2/4	50.0
Kobus e. ellipsiprymnus (Ellipsen waterbuck)	0/2	0.0
Kobus kob thomasi (Uganda kob)	0/1	0.0
Kobus megaceros (Nile lechwe)	1/2	50.0
Nesotragus moschatus zuluensis (Zulu suni)	0/1	0.0
Oryx gazella callotis (fringe-eared oryx)	1/1	100.0
Oryx gazella dammah (scimitar-horned oryx)	2/2	100.0
Oryx gazella gazella (gemsbok)	0/2	0.0
Oryx leucoryx (Arabian oryx)	0/1	0.0
Ovis orientalis gmelini (Armenian mouflon)	7/12	58.3
Saiga tatarica (Russian saiga)	0/1	0.0
Syncerus caffer (African buffalo)	0/1	0.0
Taurotragus oryx (eland)	1/4	25.0
Tragelaphus angasi (nyala)	0/1	0.0

TABLE 1. Continued.

Species (common name)	No. positive/ no. examined	% Positive
Tragelaphus euryceros (bongo)	0/1	0.0
Tragelaphus spekei (sitatunga)	0/1	0.0
Tragelaphus strepsiceros (greater kudu)	0/2	0.0
Cervus axis (axis deer)	1/12	8.3
Cervus dama (fallow deer)	2/8	25.0
Cervus duvauceli (barasingha deer)	4/8	50.0
Cervus elaphus hippelaphus (European red deer)	2/2	100.0
Cervus eldi thamin (Eld's deer)	1/2	50.0
Cervus nippon (sika deer)	1/3	33.3
Muntiacus reevesi (Reeve's muntjac)	0/1	0.0
Odocoileus hemionus (mule deer)	1/2	50.0
Capricornis crispus crispus (Japanese serow)	0/2	0.0
Total	52/183	28.4

and electrolyte replacement, anti-diarrheals, antibiotics and other supportive measures. Animal caretakers were cautioned to take care in personal hygienic practices since cryptosporidiosis is zoonotic.

It was of interest to note that *Eimeria* sp. were stained well with auramine O, making them easier to detect by this method than by routine fecal flotation methods. The occurrence of *Eimeria* sp. in only five of 183 (2.7%) cases examined indicates that this organism was probably a less important cause of neonatal diarrhea than cryptosporidia, which were present in 28.4% of clinical cases.

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