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INVASIVE ENTAMOEBAE IN PRONGHORN (*ANTILOCAPRA AMERICANA*) FROM WYOMING

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ABSTRACT: Trophozoites and cysts of an amoeba resembling *Entamoeba bovis* were recovered from soft stools of captive pronghorn fawns (*Antilocapra americana*). Chronic or intermittent diarrhea was observed in most individuals in two groups of hand-raised 1- to 8-mo-old pronghorns. Ileocecal lymph nodes were mildly to moderately enlarged. Microscopic lesions were characterized by lymphoid hyperplasia, focal necrosis and pyogranulomatous inflammation in lymph nodes and focal necrosis and diffuse nonsuppurative enteritis in ileum and cecum. In 12 of 17 fawns, trophic stages of amoebae were observed in the submucosa of the cecum and/or the cortex of the ileocecal lymph node. This is the first report of *E. bovis*-like organisms invading and causing pathological changes in the tissues of their host.

Key words: Pronghorn fawns, *Antilocapra americana*, *Entamoeba* sp., case report, diffuse nonsuppurative enteritis, ileum and cecum.

INTRODUCTION

With a few exceptions, amoebae in the genus *Entamoeba* are parasitic organisms found inhabiting various sites in the alimentary tracts of vertebrate hosts. *Entamoeba* spp. are commonly separated into four groups based on structure of trophozoites and cysts with emphasis placed on nuclear and endosomal structure and the number of nuclei found in the fully developed cyst: four nuclei are found in cysts of the *histolytica*-group; eight in the cysts of the *coli*-group; cysts are not known for the *gingivalis*-group; amoebae with uninucleated cysts are placed in the *bovis*-group of Entamoebae. Many species with uninucleated cysts from cattle, goats, sheep, swine, deer, antelope, pronghorn and other hosts have been described although morphological differentiation of species from these hosts often is not possible (Noble and Noble, 1952; Levine, 1973). When species have been described and named as distinct, the primary criterion has been a presumed host specificity. All of the species in the *bovis*-group of Entamoebae have been considered to be non-pathogenic (Levine, 1973).

Noble (1953) reported an amoeba recovered from three captive pronghorns (*Antilocapra americana*) at the now abandoned Jackson Hole Wildlife Park near Moran, Wyoming (USA). Primarily based on the host, he designated the form from pronghorn as a new species and named it *Entamoeba antilocaprae* although he conceded the fact that the new species could not be distinguished readily from *E. bovis* of cattle, *E. ovis* of sheep, *E. polecki* of goats or *E. suis* of swine.

Two species of amoebae were recovered from the rectal contents of a diarrheic white-tailed deer fawn (*Odocoileus virginianus*) necropsied at the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA) and similar amoebae were subsequently found in soft stools from live captive white-tailed deer at that institution. On morphological grounds, Kingston and Stabler (1978) identified these amoebae as *E. coli* and *E. bovis*. Trophozoites of *E. coli* measured 17 to 32 by 15 to 25 μm; octonucleate cysts measured 12 to 19 μm. Trophozoites of *E. bovis* from white-tailed deer measured 6 to 15 by 6 to 12 μm; uninucleate cysts measured 6 to 11 μm. Macroscopic or microscopic lesions were not associated with these amoebae (Kingston, 1981).

Pratt et al. (1979) described amoebae

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morphologically identical with *E. bovis* from the ceca of 13 of 14 captive elk (*Cervus elaphus nelsoni*) from the Sybille Wildlife Research Unit (Wyoming Game and Fish Department, Wheatland, Wyoming, USA). Trophozoites from elk measured 5 to 14 by 4 to 10 μm; nuclei measured 2 to 5 μm. Uninucleate cysts measured 6 to 10 μm; nuclei in these cysts measured 2 to 3 μm. Lesions attributable to amoebae were not seen in these elk.

Amoebae were recovered from the diarrheic feces of a moose (*Alces alces*) at the Sybille Wildlife Research Unit in 1977 and were considered conspecific with *E. bovis*. Uninucleate cysts measured 5 to 11 μm in diameter and nuclei measured 2 to 5 μm. The moose died some time later without recovering from the chronic diarrhea, and amoebae were present in the feces at the time of death. There was no evidence that the amoeba infection was involved in the diarrheic episodes and examination of tissues from the moose revealed no amoebae in parenteral sites. Moose constitute a new host record for *E. bovis*.

Intermittent diarrhea was seen in pronghorn fawns used in experimental studies of leptospirosis (Hoffman, 1985) at the Sybille Wildlife Research Unit in 1981 and 1982. Herein, the recovery and description of amoebae from these pronghorns and the pathologic changes associated with these parasites are documented.

MATERIALS AND METHODS

The pronghorn fawns were hand-reared on cow's milk at the Sybille Wildlife Research Center (Wyoming Game and Fish Department, Wheatland, Wyoming, USA) after having been found as orphans or captured in the wild at <1 wk of age. Sixteen pronghorns were reared in 1981 and eight in 1982. Milk was supplemented with fresh and dried sweet clover (*Meliotus* sp.), smooth bromegrass (*Bromus* sp.), sagebrush (*Artemisia* sp.) and bitterbrush (*Purshia tridentata*), and dried high-quality alfalfa hay, grain mix and fresh water. Fawns were raised on concrete-floored pens and did not have contact with other ruminants in the facility. At about 9 wk of age, 11 fawns were inoculated with *Leptospira interrogans* serovar *hardjo* as part of a study of the effects of leptospira on pronghorns. Five additional fawns were housed in contact with inoculated animals and the remaining fawns were maintained in isolation. The presence of amoebae in pronghorn was not correlated with experimental group; in the remainder of this report animals are discussed without reference to the leptospirosis study.

Feces were examined in saline or iodine wet-mount preparations by light and phase contrast microscopy. Permanent preparations were wet-fixed in hot Schaudinn's solution and stained with Gomori's trichrome or iron hematoxylin. Photomicrographs were made at 450 and 1,000× magnifications of fresh and fixed specimens and measurements were made using a calibrated filar micrometer.

Fawns were necropsied as soon as possible following death or euthanasia at approximately 12, 14, 15, 31 and 33 wk of age. In 1981, two fawns were euthanized at approximately 14 wk of age because of weakness and persistent diarrhea; blindness and circling also occurred in one of the animals. These animals had been treated with antibiotics (Penicillin G Benzathine and Penicillin G Procaine, Pfizer, Agricultural Division, New York, New York 10017, USA). Six pronghorn were not killed. Sections of brain, thyroid gland, adrenal gland, pituitary gland, lung, liver, heart, kidney, mesenteric lymph nodes, ileum, cecum, spiral colon and eye were collected from most animals, fixed in 10% neutral buffered formalin or Zenker's solution, embedded in paraffin, tissue sections cut at 5 μm, and stained with hematoxylin and eosin.

Depending upon the presence of gross lesions, brain, liver, lung, mesenteric lymph nodes, cerebrospinal fluid (CSF), bile or feces, were cultured by standard techniques for pathogenic bacteria (Carter, 1984).

RESULTS

From late June to late December soft to normally pelleted stools from eight pronghorn fawns were positive for trophozoites and cysts of amoebae; stools from pronghorn yearlings examined at various times from May to January were also positive for amoebae (Fig. 1). Equal numbers of male and female pronghorns were positive for amoebae. Amoebae were not present consistently in stools from individual animals. Live trophozoites were irregular to oval in shape, had a thin, broad hyaline pseudopod and measured between 8 and 11 μm, ($\bar{x}$ = 10 μm), prominent nuclei measured between 3.0 and 3.6 μm. Live cysts aver-

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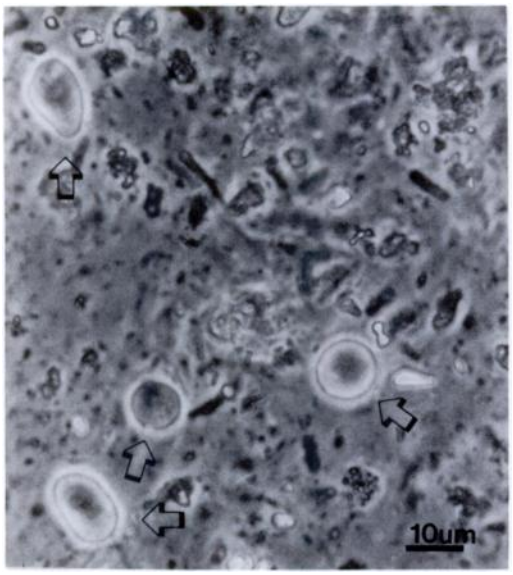


FIGURE 1. Amoebae (arrows) in feces from a pronghorn.

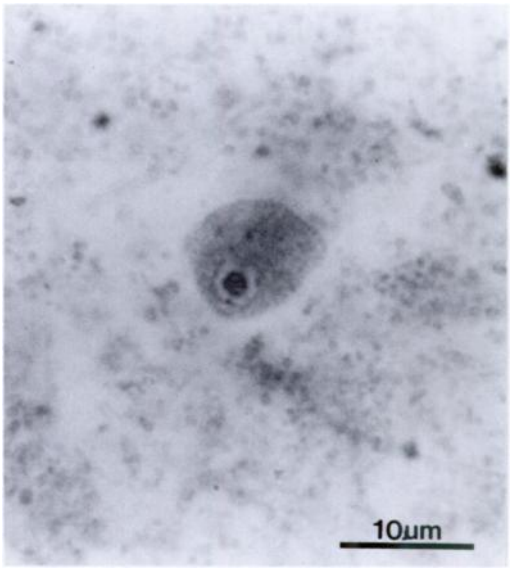


FIGURE 2. Fixed trophozoite of amoeba in feces from a pronghorn.

aged 9.4 µm, the single nucleus averaged 3.6 µm. Fixed trophozoites (Fig. 2) measured 9.2 to 10.7 by 7.9 to 8.7 µm ($\bar{x}$ = 9.8 by 8.3) with nuclei 3.1 to 3.6 µm ($\bar{x}$ = 3.2 µm) with a central, small punctate endosome surrounded by a periendosomal cloud. Fixed cysts (Fig. 3) measured 7.6 to 10.2 by 7.4 to 9.9 µm ($\bar{x}$ = 8.8 by 8.6) in diameter with nuclei 3.1 to 4.3 µm ($\bar{x}$ = 3.7 µm). Size, shape, the single cystic nucleus with its small, punctate central endosome, the distribution of periendosomal granules, the marginal displacement of the nucleus by a large glycogen vacuole and rounded chromatoid bars seen in these forms from pronghorn are consistent with the descriptions of *E. bovis* from ruminants in North America (Noble and Noble, 1952; Noble, 1953; Levine, 1973) and thus are considered conspecific with that species.

At necropsy, several fawns were in poor to fair condition as judged by body size and amount of subcutaneous and visceral fat; others were in good to excellent condition. All fawns had mildly to moderately enlarged mesenteric lymph nodes, especially the ileocecal lymph nodes. In many fawns, intestinal contents were soft to fluid. One 14-wk-old fawn, killed in 1981 because of weakness, diarrhea and signs of central nervous system disease, had multiple 1 to 2 cm yellow-grey foci on capsular and cut surfaces of the liver.

Microscopically, 14 of 17 fawns had

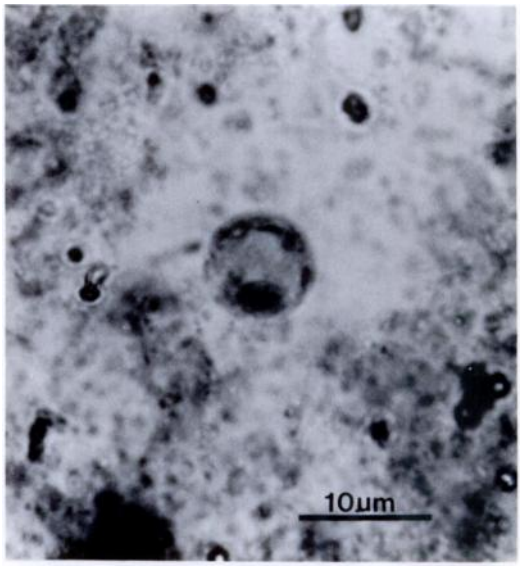


FIGURE 3. Fixed amoeba cyst in feces from a pronghorn.

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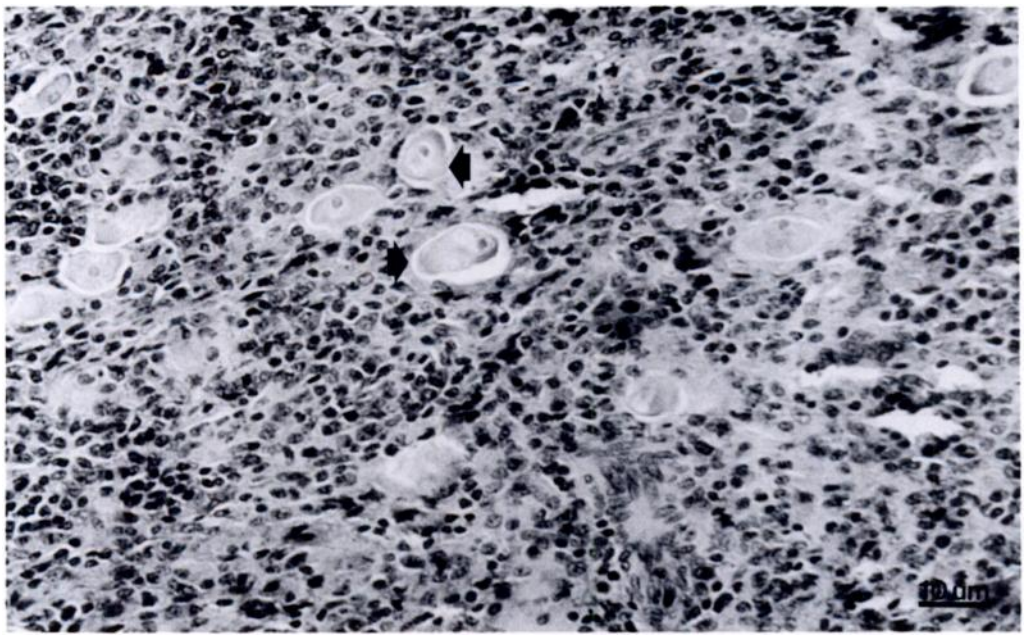


FIGURE 4. Trophozoites (arrows) of amoebae in the cortex of an ileocecal lymph node from a pronghorn fawn.

chronic enteritis, characterized by infiltration of lymphocytes and plasma cells into the lamina propria of the distal small intestine and cecum. In 12 fawns, organisms identified as trophozoite stages of amoebae were present in the submucosa of the distal ileum and cecum. Amoebae, measuring 29 to 38 by 20 to 22 μm, were generally surrounded by lymphocytes, plasma cells and macrophages; a few neutrophils were present. In most cases, small foci of necrosis and pyogranulomatous inflammation, in the absence of identifiable amoebae, also were present in the submucosa. Lesions suggestive of viral infection of the intestine were not observed (Barker and Van Dreumel, 1985). Inflammation of the afferent lymphatics occurred in three fawns. The ileocecal lymph nodes of 11 animals contained lesions which varied from lymphoid hyperplasia to focal necrosis and suppurative or pyogranulomatous inflammation. Amoebae were observed in the ileocecal lymph nodes (Fig. 4) of two fawns. Lesions in the liver of one fawn were coagulative; adjacent vessels were thrombosed. A few forms resembling amoebae were present in the liver. This animal also had suppurative meningoencephalitis; amoebae were not observed in multiple sections of the brain or meninges.

Cultures of feces did not yield significant pathogens (*Escherichia coli*, *Pseudomonas putrefaciens*). *Neisseria* sp. was cultured from the lung of one animal. Cultures of mesenteric lymph nodes yielded *E. coli* or no bacterial growth. Bacterial growth did not occur in cultures of liver, brain, CSF or bile.

DISCUSSION

Prior to this report, no *Entamoeba* sp. has been shown to be invasive/pathogenic to its host except *E. histolytica*, and it is only intermittently so (Levine, 1973). *Entamoeba suis* has been found associated with necrosis in sections of the colon of swine infected experimentally with virus of hog cholera (Ratcliff, 1934). Our finding of invasive amoebae of an *E. bovis*-type in small intestine, cecum and lymph node of pronghorn associated with inflammation

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and necrosis parallels, except for the small intestine, the classic and well-known pattern of pathogenesis seen in *E. histolytica* infections (Elsdon-Dew, 1968). Such lesions associated with amoebae in pronghorn fawns were not recognized at Sybille prior to 1981 nor since 1982 and the occurrence of such lesions is unexplained. It is not known if free-ranging pronghorn may harbor this parasite. The absence of lesions in hosts such as white-tailed deer, elk and moose infected with amoebae is also unexplained.

We have referred to the species of amoebae found in white-tailed deer, elk, moose and pronghorn as *E. bovis*; all of these forms are morphologically indistinguishable from the bovine species of amoeba. It can be argued that the species of amoeba from pronghorn should be referred to as *E. antilocaprae* as described by Noble (1953), but that species was not clearly separated morphologically from *E. bovis* nor from other species of *Entamoeba* from ruminants and some non-ruminants (Noble and Noble, 1952; Noble, 1953). It seems preferable at this time not to name new species but to retain the uninucleate amoebae from these ruminants as *E. bovis* until such time that further studies, especially involving cross-transmission, host resistance and biochemical differences, are completed. Further, we recommend that investigators studying the parasites of wild ruminants should examine feces and tissues from such hosts for amoebae with the ultimate purpose of defining pathogenesis, host specificity, and species identity of these parasites.

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