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HEPATIC LESIONS ASSOCIATED WITH *COOPERIOIDES HEPATICAE* (NEMATODA: TRICHOSTRONGYLOIDEA) INFECTION IN IMPALA (*AEPYCEROS MELAMPUS*) OF THE KRUGER NATIONAL PARK

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ABSTRACT: Intrahepatic biliary lesions were observed in two of 12 lambs, seven of 12 yearlings and 10 of 25 adult impala (*Aepyceros melampus*) surveyed in the Kruger National Park, Republic of South Africa. Lesions were associated with the nematode *Cooperioides hepaticae*, a trichostrongyloid parasite that inhabits the bile ducts of impala, and ranged from a mild chronic-eosinophilic cholangitis to foci of florid hyperplastic cholangitis with duct ectasia. The latter almost always contained viable worms and, after the worms died, the lesions appeared as foreign-body granulomas. Infection was acquired early in life; severe lesions were seen most frequently in yearlings. Adults were less severely infected, which suggested an acquired immunity. Although the incidence of infection was high, cooperiiasis did not appear to be a serious herd-health problem at the time of this study.

Key words: Intrahepatic biliary lesions, pathology, nematode, *Cooperioides hepaticae*, impala, *Aepyceros melampus*, field survey.

INTRODUCTION

During a study of parasitic infections and pathologic changes in free-living impala (*Aepyceros melampus*) of the Kruger National Park (KNP), Republic of South Africa, it was noted that a large number of the antelope had varying degrees of intrahepatic biliary lesions due to infection by a trichostrongyloid nematode (*Cooperioides hepaticae*). Species of the genus *Cooperioides*, with the exception of *C. hepaticae*, are intestinal parasites found in domestic sheep and several types of antelope in eastern and southern Africa (Daubney, 1933; Messer, 1952; Round, 1968). Like members of the genus *Cooperia*, *C. hepaticae* are generally considered of minor pathologic significance unless present in large numbers in combination with other trichostrongyles, and/or in association with a poor nutritional condition. *Cooperioides hepaticae* is the only species of the genus with adults occupying an extraintestinal location. Moreover, the species appears to be the only trichostrongyloid that inhabits tissues other than those of the gastrointestinal tract (Daubney, 1933; Ortlepp, 1938; Soulsby, 1968).

We have conducted a systematic study of endoparasite and ectoparasite frequencies and numbers and associated pathologic changes of impala in the southern part of the KNP. Impala are the most abundant antelope in the eastern Transvaal. It has become evident from our work that *C. hepaticae* infection is very common among these impala and can cause substantial lesions. This report describes gross and microscopic lesions produced by *C. hepaticae* in the livers of impala and discusses the host response to the infection. Other aspects of this parasite such as taxonomic morphology and life cycle are not addressed except in acknowledgement of the facts that the taxonomy of the genus *Cooperioides* has been reviewed (Gibbons,

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TABLE 1. Intensity of *Cooperioides hepaticae* infection in impala by sex and group.

Age group	Number of animals			Mean intensity (range)	
	Female	Male	Total	Female	Male
Lambs (0–6 mo)	3	9	12	67 (0–265)	71 (6–295)
Yearlings (12–18 mo)	3	9	12	230 (15–680)	226 (10–683)
Adults (over 24 mo)	7	18	25	32 (0–230)	31 (8–225)

1978) and that the life cycle of *C. hepaticae* has not been described but is thought to be direct.

MATERIALS AND METHODS

All impala in this study were collected at the Kruger National Park, Republic of South Africa (25°12′ to 24°24′S and 31°36′ to 32°02′E). The details of the park habitat are described briefly by Krecek et al. (1987).

Impala of the appropriate age were selected at random, shot through the neck with a high caliber rifle and then exsanguinated by severing the major vessels in the neck. The majority of the impala studied were males from three age groups—1 to 6 mo (lambs), 12 to 18 mo (yearlings), and over 2 yr (mainly prime adults). A smaller number of females of similar ages were also surveyed.

Tissues taken for histopathologic studies were fixed in 10% neutral buffered formalin and prepared and stained according to commonly accepted methods. Hematoxylin and eosin as well as Masson's trichrome stains were applied to the sections studied. The entire liver of each animal was macerated and the number of *C. hepaticae* tabulated for each. Liver sections containing *C. hepaticae* were deposited at the Armed Forces Institute of Pathology (Washington, D.C. 20306, USA; Accession Number 2168059) and by the U.S. National Parasite Collection (Animal Parasitology Institute, USDA, Beltsville, Maryland 20705, USA; Accession Number 80361).

RESULTS

The data on the intensity of infection in the impala are summarized in Table 1.

The general physical appearance of the animals ranged from poor to very good. Those in poor condition were usually yearlings or young adults, and necropsy of these animals revealed heavy parasite infections, with the lesions of *Pneumostrongylus calcaratus* in the lungs and of *C. hepaticae* in the liver being most notable. Impala <6 mo old did not have gross lesions associated with either of these parasites. Prime adults were generally in good condition, although many had macroscopic evidence of both lungworms and biliary cooperiiasis, but to a lesser degree than did the yearlings. The prevalence of infection by both parasites appears to be the same for male and female impala.

Lesions associated with *C. hepaticae* were limited to the liver. In mildly infected animals (those with <100 nematodes in the liver), macroscopic changes were subtle, consisting of a slight prominence of the portal tracts within the liver. These areas offered increased resistance when the parenchyma was incised, suggesting mild portal fibrosis. In several animals, the bile duct tapeworm *Stilesia hepatica* was present along with *C. hepaticae*. Gross lesions specific for *C. hepaticae* were observed in heavily infected impala (those having >200 nematodes in the liver); these animals were most often yearlings. In addition to prominent portal fibrosis, focal yellow-white spherical to ellipsoidal nodules were seen within the liver. Nodules were as large as 1 × 2 cm and often could be seen at the surface of the liver but were present throughout the parenchyma as well. A heavily infected liver would contain ≥eight nodules, which, when incised, revealed a substantial fibrous capsule encompassing a greatly thickened and dilated bile duct containing many reddish nematodes and a cloudy fluid (Fig. 1). Nodules of similar size were occasionally found to be firm and, on incision, to contain a caseous material that had a gritty consistency. Nematodes were not seen in these lesions.

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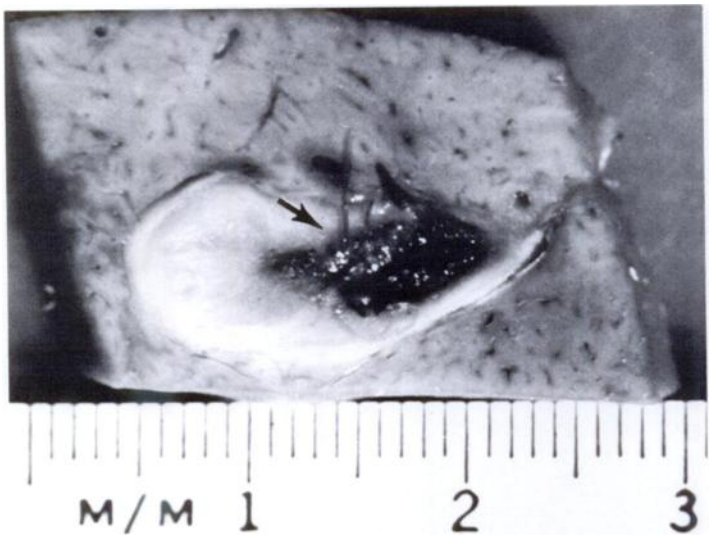


FIGURE 1. Sagittal section through an active *Cooperioides hepaticae* nodule. There are many reddish worms (arrow) in the greatly thickened and sacculated bile duct. Formalin-fixed specimen.

Microscopically, animals with early, or mild, infections showed a chronic-active eosinophilic cholangitis with an increase in periportal fibrous tissue. Eosinophils and plasma cells were the predominant inflammatory cells in the lamina propria, and the biliary epithelium was often noted to be slightly hyperplastic. Portal veins were frequently dilated and congested. In addition to these changes, severely infected animals had focal areas of moderate to extreme cholangiectasia with florid biliary epithelial hyperplasia often resulting in papillary structures which extended into the sacculated bile duct lumens. Within these lumens were adult *C. hepaticae*, their eggs, and mucoid material mixed with cellular debris (Fig. 2). The epithelium at the tips of the papillary structures often showed a marked squamous metaplasia; and eosinophils, as well as mononuclear cells with variably sized eosinophilic globules in their cytoplasm, were frequently seen within the epithelium. Remnants of both these cells also could be seen in the luminal debris. Connective tissue beneath the epithelium was infiltrated by many plasma cells and eosinophils, and these inflammatory cells, in addition to aggregates of lymphocytes, were prominent in the surrounding fibrous tissue. The hepatic parenchyma adjacent to these nodules appeared compressed, attesting to the expansile nature of the lesions. The firm nodules having no nematodes consisted of a central core of eosinophilic material containing mineralized debris and were surrounded by a zone of multinucleated giant cells and macrophages. At the periphery were inflammatory cells including eosinophils and plasma cells, but macrophages and lymphocytes predominated, and the development of lymphoid follicles was noted (Fig. 3). Biliary epithelium was not evident in these lesions.

DISCUSSION

The genus *Cooperioides* was originally described by Daubney (1933), who acknowledged the close relationship between it and the genus *Cooperia*. Various species have been recovered from the intestines of domestic sheep, springbok (*Antidorcas marsupialis*), Thomson's gazelle (*Gazella thomsoni*) and impala (*Aepyceros melampus*) (Daubney, 1933; Round, 1968); however, Ortlepp (1938) described *Cooperioides hepaticae* found "in small nod-

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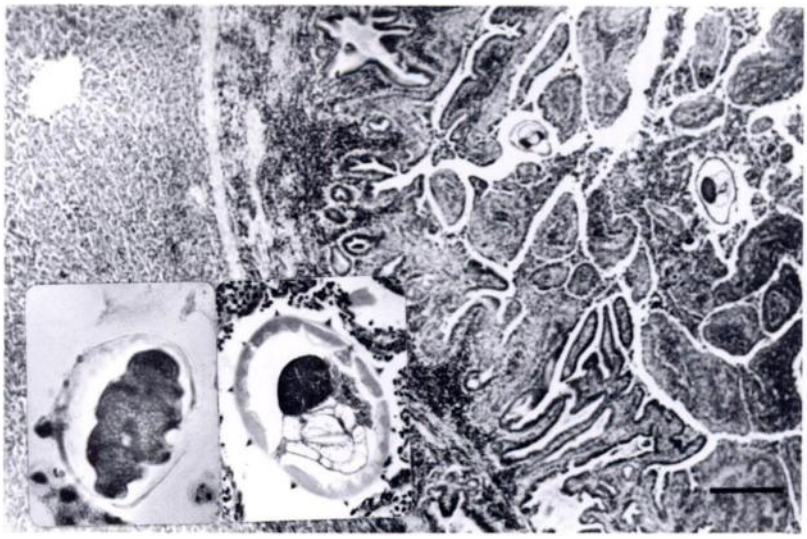


FIGURE 2. An active parasitic nodule. Note the papillary hyperplasia of biliary epithelium with superficial squamous metaplasia and the nematodes in cross section. H&E (bar = 1 mm). Insets: left, *Cooperioides hepaticae* egg measuring 30 × 40 μm; right, mature *C. hepaticae* in cross section.

ules in the terminal portions of the bile ducts" of an impala from the northern Transvaal. Messer (1952) described an identical nematode from the intrahepatic bile ducts of an impala in the eastern Transvaal. He referred to this parasite as "*Cooperia hepaticae*" and briefly described the pathologic changes associated with it (Messer, 1952). Mugera (1969) provided brief descriptions of the lesions produced by *C. hepaticae* and the tapeworm *Stilesia hepatica* in the liver of impala but it appears that he confused the lesions produced by the two species. Basson et al. (1971) mentioned *Cooperioides hepaticae* as a parasite of the biliary ducts of impala from the KNP, identified it as a "bile duct hookworm" and grouped it with true hookworms such as *Grammocephalus clathratus*, the bile duct hookworm of the African elephant (*Loxodonta africana*).

Gross and microscopic observations of *C. hepaticae* lesions and the intensity of infection suggest that there is a progression of events. An initial infectious phase proceeds through the development of active parasitic nodules and peak intensity. This is followed by subsequent destruction of adult nematodes and resolution of nodules. Mild eosinophilic cholangitis with a resulting increase in portal connective tissue occurs during the initial stages. These changes are first recognized at about 6 mo of age and probably reflect the migration of larvae and/or adult worms up the biliary tree. Similar lesions could occur as a result of the bile duct tapeworm *S. hepatica*; however, the occurrence of cholangiectasia and nodule formation is specific for biliary cooperiiasis. Although initial infection probably occurs early in life, substantial macroscopic lesions (parasitic nodules) are not observed until the animals approach 1 yr of age. Active parasitic nodules incite an immune response characterized by large numbers of plasma cells and eosinophils. Eosinophils and mononuclear cells with eosinophilic cytoplasmic globules were seen within the epithelium and in the luminal debris of parasitic nodules, and it seemed that both types of cells, as well as the plasma cells in the lamina propria, were intimately involved in the host response to the parasites. The cytoplasmic globules in the mononuclear cells appeared similar to Russell bodies, and their

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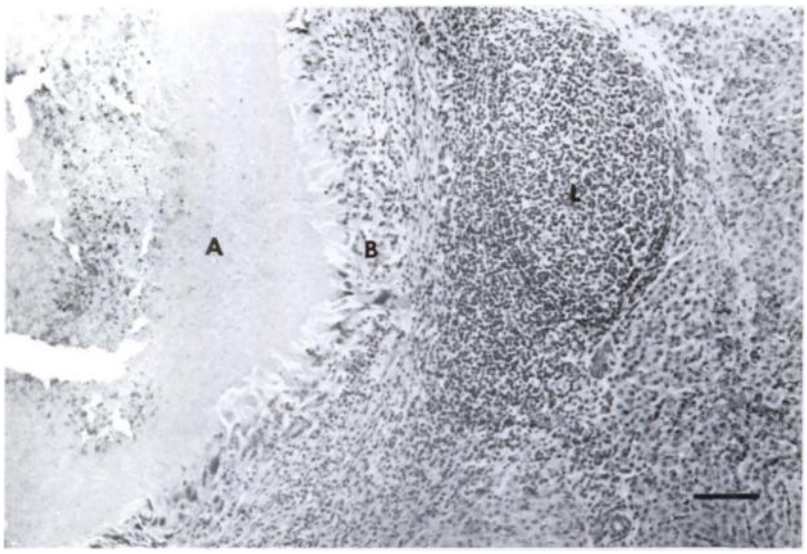


FIGURE 3. A resolving parasitic nodule with central core of partially mineralized eosinophilic debris (A), a zone of multinucleated giant cells and macrophages (B), and a peripheral zone predominantly of lymphocytes and macrophages with lymphoid follicle development (L). Note that biliary epithelium is not evident. H&E (bar = 250 μm).

possible role in the transport of antibody across the epithelium is the subject of continuing study. It has been demonstrated recently that eosinophils, in the presence of specific antibodies, can function as effector cells in the destruction of metazoan parasites (Zucker-Franklin, 1978). Once the adult worms are dead, the lesions appear to resolve as foreign-body granulomas. Macrophages and multinucleated giant cells predominate and lymphoid follicles are formed. The hyperplastic biliary epithelium observed in active nodules is obliterated in the process of resolution. Although many adult impala are infected, these tend to have fewer active parasitic nodules, and the prevalence of infection is lower than that for the yearlings. One can deduce from these findings that most adult impala acquire at least a partial immunity.

The florid biliary hyperplasia and metaplasia seen in active nodules is of interest. Apparently, biliary stimulation results from some parasite-associated factor(s) similar to that described in trematode-induced biliary hyperplasia observed in domestic ruminants (Isseroff et al., 1977).

Cooperioides hepaticae infection is one of the most common extraintestinal parasitic infections of impala in the KNP. Substantial lesions can accrue as a result of severe infection, particularly in yearlings, and it is highly probable that such lesions adversely affect the health of animals. However, it is doubtful whether *C. hepaticae* alone is a primary factor limiting the population of the large impala herds of the eastern Transvaal. Impala are the most numerous antelope in the KNP, despite a high prevalence of infection. Conversely, it is reasonable to assume that biliary cooperiiasis, in concert with lung worms, gastrointestinal helminths and ectoparasites could cause excessive losses in certain environmental settings. The depletion of impala herds in the eastern Transvaal during the dry seasons of 1949 and 1950 is an example (Messer, 1952). Game farmers and conservationists should be familiar with the conditions that tend to en-

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hance these various parasitic diseases so that appropriate preventive measures can be applied when feasible.

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