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SUSCEPTIBILITY OF PEKIN AND MUSCOVY DUCKS TO *HAEMOPROTEUS NETTIONIS*

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ABSTRACT: Domestic muscovy (*Cairina moschata*) and pekin (*Anas platyrhynchos*) ducklings were exposed to or injected with homogenates of *Culicoides* flies collected at White Pine, Michigan. Gametocytes of *Haemoproteus nettionis* were detected in both species of ducks at 16 days post-exposure. *Culicoides downesi* was indicated as a vector of *H. nettionis* in northern Michigan. Blood infections occurred with a higher prevalence and reached a higher intensity in muscovy ducks. Endogenous tissue stages of *H. nettionis* were located in endothelial cells of the lung and occasionally in the heart and spleen. Attempts to infect domestic ducklings by blood or tissue inoculations from wood ducks (*Aix sponsa*) infected with *H. nettionis* were not successful, even though schizonts of *H. nettionis* were seen in these wood ducks at necropsy. No morbidity or mortality was observed in any ducks infected with *H. nettionis*.

INTRODUCTION

Infections by *Haemoproteus* are typically nonpathogenic to their avian hosts, although occasional acute cases have been reported for *H. columbae* by Markus and Oosthuizen (1972) and for *H. lophortyx* by O'Roke (1930). Infections of North American waterfowl (Anatidae) are considered to comprise a single species, *H. nettionis* (Johnston and Cleland, 1909) as reviewed by Williams and Bennett (1980). Infections of *H. nettionis* are generally nonlethal; however a report of mortality of muscovy ducks in Ontario was attributed to infections by *Haemoproteus* by Julian and Galt (1980). Recently Kucera et al. (1982) have also reported fatal haemosporidian infections in muscovy ducks in Europe. The absence of blood stages in muscovies dying of schizont-congested lungs and in muscovies which did not succumb to infectious challenge (Julian and Galt, 1980) raises some doubt as to the identity of this parasite. The present study reports nonpathogenic infections of *H. nettionis* in domestic pekin and muscovy ducks following controlled infectious challenge. A description of tissue schizogony of *H. nettionis* is reported for the first time from ducks with verified blood infections.

MATERIALS AND METHODS

Domestic ducklings were purchased from Abendroth's Waterfowl Hatchery, Waterloo, Wisconsin 53594, USA, and maintained in vector-free facilities at Northern Michigan University or in outdoor cages covered with *Culicoides*-proof screening (60-mesh nylon). Ducks were bled every 2–3 days from puncture of a leg vein. Blood parasites were counted during 5 min examination (450×) of thin smears stained in 0.1 M buffered Giemsa's stain (pH 7.2), during which time approximately 15,000 red blood cells (RBC's) were scanned. Parasitemias were expressed as number of parasites per 1,000 RBC's. For histological examination, tissues were fixed with 10% formalin in Millonig's phosphate buffer (300 mosmolar). Paraffin sections (4–5 μm) were stained with hematoxylin-eosin (HE) or with Giemsa-colophonium (GC) as described by Garnham and Bray (1962).

Two wood ducks infected with *H. nettionis* were obtained from Hiram Biological Station, Ohio 44234, USA, in early May 1982. The wood ducks were housed in vector-free facilities for 2 wk during which time blood smears revealed consistently high (1–5% of RBC's infected) parasitemias of *H. nettionis*. To evaluate possible latent infections of *Plasmodium*, 2 cc of blood from each wood duck were injected intraperitoneally (i.p.) into two pekins and two muscovy ducks. To evaluate experimental transmission

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of *H. nettionis* by tissue or blood inoculation, the wood ducks were used for two separate experiments. One-cm³ pieces of lung, spleen, heart, liver, and kidney were homogenized with a glass tissue grinder in Hanks' Balanced Saline (HBS), pH 7.2 at 22 C and filtered through gauze. For each wood duck a separate set of 1–3-day-old ducklings received 2-cc i.p. injections of pooled tissue homogenates (4 pekin, 4 muscovy), 1-cc intramuscular (i.m.) injections of citrated whole blood (2 pekin, 2 muscovy), or 2-cc i.m. control injections of HBS (2 pekin, 2 muscovy).

Naturally acquired infections were studied by exposing ducks to suitable vectors at White Pine, Michigan 49971, USA, from 1 to 3 July 1982. During the daylight hours (7:00 a.m. to 11:00 p.m.) cages were covered with 60-mesh nylon screen to prevent infections with *Leucocytozoon simondi* which is transmitted by black flies (Fallis and Wood, 1957). Vectors were collected with an aspirator between 11:00 p.m. and 2:00 a.m. from around lights placed near exposed ducklings. Flies were maintained at 22–25 C overnight. Pools of about 200 specimens of *C. downesi* were homogenized in uninfected citrated duck blood and injected IM into each of three 1–3-day-old pekin and muscovy ducklings. Control ducklings received equal volumes of HBS or of uninfected duck blood. Additional control ducks received no exposure to vectors or injections and were housed with experimental ducklings.

Representative slides of *H. nettionis* in thin blood films and schizonts in tissue sections from wood ducks (#91933) and muscovy ducks (#91932) have been deposited in the International Reference Centre for Avian Hematozoa, Memorial University of Newfoundland, St. John's Newfoundland, A1B 3X9, Canada.

RESULTS

Mature gametocytes of *H. nettionis* were typically halteridial (Fig. 1) and conformed to descriptions by Williams and Bennett (1980). Mature forms which completely encircled the host nucleus (circumnuclear) were also observed. No infections resulted in any of the 24 ducklings inoculated with tissue or blood from wood ducks, although schizonts of *H. nettionis* were present in the wood ducks at necropsy. In addition, no *Plasmodium* infections were detected in any ducks used in this study. Among those ducks exposed to vectors, no infections of *Leucocytozoon* occurred.

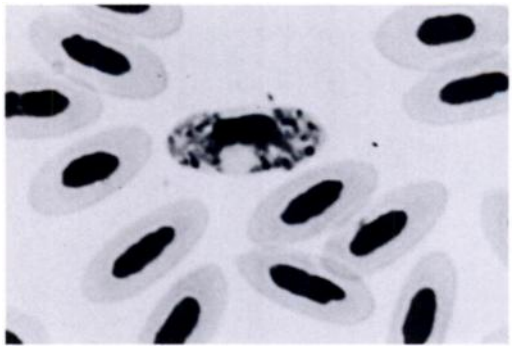


FIGURE 1. Mature macrogametocyte of *Haemoproteus nettionis*. ×1000.

Mature gametocytes of *H. nettionis* developed in one of six pekins and five of six muscovy ducks which were exposed to night feeding *Culicoides*. Gametocytes first appeared 16 days after initial exposure and mature forms were observed at 22 days after exposure in both species of ducks. Infections of *H. nettionis* were detected in all six ducks inoculated with pooled specimens of *C. downesi*. The level of parasitemia in muscovy ducks in the present study was considerably higher than that observed in pekin ducks given equal infectious challenge. A dramatic fall in circulating parasites was observed between 16 and 22 days post exposure in both groups of muscovy ducks (Fig. 2). This decline in parasitemia was apparently due to a rapid clearance of infected cells. Mature gametocytes seen in blood smears from muscovies appeared normal in morphology.

Tissues from ducks infected with *H. nettionis* showed moderate infiltration with lymphocytes and granulocytes and an accumulation of malarial pigment and cellular debris in tissue macrophages. Schizonts of *H. nettionis* detected in Giemsa stained paraffin sections of muscovy and wood ducks were similar in form and location. Schizonts were not detect-

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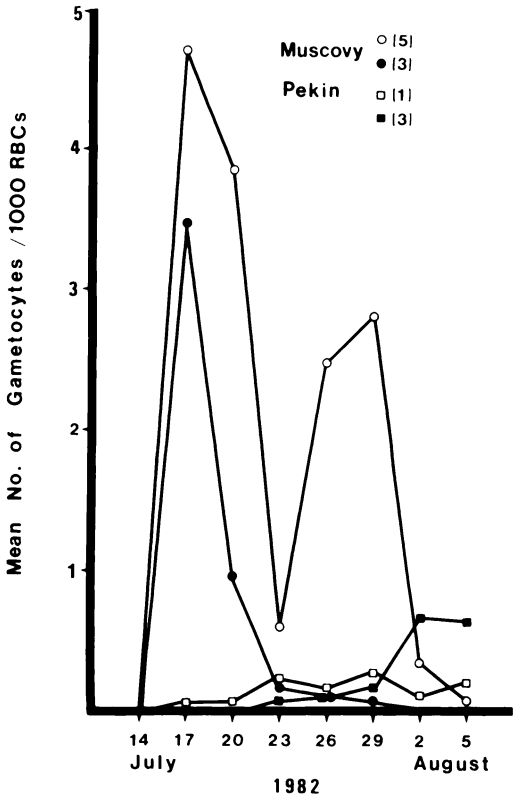


FIGURE 2. Mean parasitemias of *Haemoproteus nettionis* in ducks exposed to (solid symbols) or injected with (open symbols) *Culicoides downesi* in northern Michigan. Numbers of positive ducks are indicated in parentheses.

able in sections of pekin tissues, due probably to the low level of infections. Schizonts located in endothelial cells were seen primarily in the lungs, but also in the heart and spleen. Small schizonts contained vacuoles as well as densely staining merozoites. Mature schizonts were typically oval in shape and contained lighter staining merozoites (Fig. 3). Cytomeres were not evident and branching or elongate schizonts were seldom seen. Schizonts were highly variable in size and ranged from 3–10 μm wide by 6–30 μm long ($n = 50$) and host cell pyknosis was evident in larger schizonts. Merozoites ranged from 0.6 to 1.1 μm in diameter and showed dark and light areas of staining with Giemsa

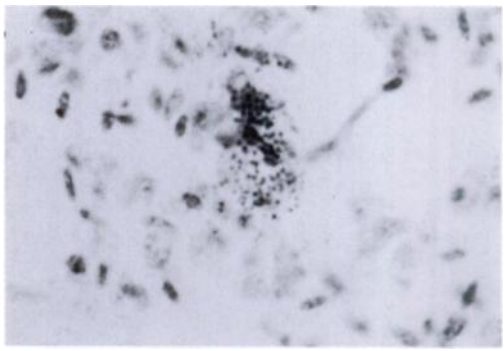


FIGURE 3. Giemsa stained section of lung tissue containing mature schizont of *Haemoproteus nettionis*. ×430.

corresponding to nuclear and cytoplasmic regions.

We have also had the opportunity to examine Giemsa-stained histoslides of muscovy ducks which died of schizont-congested lungs in Ontario. Schizonts were seen to contain two distinct populations of merozoites. A 1-μm form was described previously from HE stained sections by Julian and Galt (1980). In addition, elongate merozoites 1–2 μm by 2–3 μm were evident in most schizonts. These latter merozoite forms were not evident in the initial HE stained slides due to a very light staining of cytoplasm.

DISCUSSION

Haemoproteus nettionis was the only hematozoan parasite observed in repeated blood smears made during this study. Subinoculations of blood from ducks infected with *H. nettionis* into recipient ducklings confirmed the absence of latent *Plasmodium* infections. *Leucocytozoon simondi*, which occurs in ducks exposed at White Pine, Michigan (Desser et al., 1978), was not observed in experimental birds housed within 60-mesh nylon netting during the daylight hours.

Previous reports (Huff, 1942; Desser et al., 1968; Bennett et al., 1974) have indicated a spring relapse pattern for *H. nettionis*. Relapses of *H. nettionis* were ob-

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served in three domestic geese (*Anser* sp.) in March and lasting through May, after a negative blood phase that had persisted from October through February (Sibley, 1982). Thus, the high parasitemias of *H. nettionis* observed in wood ducks during early May were likely due to relapses of infections acquired during the previous year. The *Culicoides* vectors of *H. nettionis* typically emerge and become common in late June or early July (Jamnback, 1960) as was observed in localities of this study.

Low infectivity of the merozoites of *Haemoproteus* spp. in tissue inoculations has been reported by Coatney (1933) and O'Roke (1930). Our results confirm these observations for *H. nettionis*, although failure to infect any of 16 ducks injected with macerated tissues was unexpected in view of the confirmation of tissue schizonts in tissues of donor wood ducks at necropsy. The noninfectivity may have been due to few mature merozoites present at the time of death or to merozoite death during the inoculation procedure. In contrast, sporozoite inoculations produced high rates of infection (6 of 6 ducklings). Similar infectivity of *H. columbae* sporozoites has been reported by Rendtorff et al. (1949).

Culicoides downesi was first confirmed as the vector of *H. nettionis* in Ontario by Fallis and Wood (1957). This report extends the known range of *C. downesi* as a vector of *H. nettionis* to northern Michigan, as established by infections seen in ducks exposed to or injected with this species. The intensity of infections in ducks observed in this study was similar to that reported by Herman et al. (1971) with 0.1 to 5% or RBC's infected. The relatively low levels of infections in pekin ducks relative to a report using this species as a sentinel (Herman and Bennett, 1976) were an indication that the level of exposure in the present study was rather low. Herman and Bennett (1976) reported infections as high as 15% of RBC's in ducks exposed for up to 5 wk. The relatively low levels of infections seen in pekins in the present study reflect the much shorter exposure period of 3 days used here.

The prevalence and intensity of infections was significantly higher in muscovy ducks, although no visible illness was noted in either species of duck. Even though muscovy ducks showed indications of being more susceptible to development of blood stages, many of these forms were rapidly cleared from the circulation by the time parasites appeared mature (22 days postexposure). The presence of mature gametocytes in circulating blood from muscovy ducks indicated that the life cycle of *H. nettionis* proceeded normally in this host. We were not able to conduct studies on the infectivity of gametocytes from the different duck hosts.

Tissue stages of *H. nettionis* are described here for the first time from wood ducks and muscovy ducks with confirmed single species blood infections. Similar stages were seen at a low density in lung tissue of infected domestic geese (Sibley, 1982). Schizonts of *H. nettionis* differed from previous descriptions of haemoproteids (Khan and Fallis, 1969; Ahmed and Mohammed, 1973) in being uniformly smaller, containing smaller merozoites (average 1 μm), and rarely showing branching or elongation; cytomeres were not evident. Staining reactions of merozoites during maturation were similar to previous descriptions of *H. columbae* by Bradbury and Gallucci (1972). Schizonts appeared similar in primary infections (muscovy ducks) and relapse infections (wood ducks). Schizonts occurred in endothelial cells of several organs with a distinct concentration in lung tissue, similar to the pattern described for *Parahaemoproteus fringillae* by Khan and Fallis (1969).

The present study does not support the conclusion that *Haemoproteus* causes mortality in muscovy ducks. In addition to the lack of pathogenicity, *H. nettionis*

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as described here differed from the organism infecting muscovy ducks in Ontario in schizont morphology, ease of experimental transmission, and gametocyte development. Schizonts described here were smaller in overall size and contained only spherical (1 μm) merozoites while schizonts in lung tissue of muscovy ducks from Ontario contained both spherical (1 μm) and oval (1–2 by 2–3 μm) merozoites. Experimental transmission of *H. nettionis* by blood or tissue inoculations was not produced in the present study, although moderate infections resulted in muscovy ducks from natural exposure to vectors. In contrast, Julian and Galt (1980) reported high infectivity by blood inoculation of an organism which did not complete gametocyte development in muscovy ducks. This pattern of pathology prior to gametocyte development has also been reported by Fleishman et al. (1968) from African penguins (*Spheniscus demersus*) infected with *Plasmodium elongatum*.

The present study indicates that infections of *H. nettionis* are nonpathogenic in pekin and muscovy ducks. At higher intensities anemia, typical of heavy hematozoan infections, may become evident.

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BOOK REVIEW . . .

Veterinary Biology and Medicine of Captive Amphibians and Reptiles, Leonard C. Marcus. Lea & Febiger, 600 Washington Square, Philadelphia, Pennsylvania, USA. 1981. 239 pp. $25.50 US.

This relatively new book supplies a well organized and concise overview of poikilotherm husbandry, management, and medicine. The text is divided into only three chapters, and fortunately two of these deal with the basic biology and requirements of amphibians and reptiles.

The first chapter deals with practical anatomy and physiology, and as with any basic science, is a necessary primer for understanding and treating disease problems in these groups. The material is a concise summary of pertinent herpetology and could serve as a basic classroom text in vertebrate zoology or related exotic animal medicine courses.

Chapter 2 is also a summarization, dealing with husbandry and care of normal specimens prior to morbidity. This chapter is somewhat brief, but adequately covers the principles of captive care. Some subtle points of captive care are also noted, and these fine points relate to the experience of the author. Medical and surgical principles also are covered briefly, but specific techniques and indications for coelomic surgery are not included.

Specific disease problems are presented in Chapter 3 and are outlined well. Some space was utilized poorly with technical schematics which do not fit well with the basic theme. The pathological aspects are presented from a pathologist's perspective and histopathological photomicrographs may intimidate the novice herpetologist. Zoonoses are also covered.

For what the text lacks in detail to satisfy professional herpetologists and allied professionals, the bibliography compensates as a thorough compilation of the current literature prior to publication. Unfortunately, some timely information regarding paramyxovirus could not be included prior to publication. A list of sources for obtaining current relevant information would have been a useful inclusion, but these can be gleaned from the references.

For the herpetologist and/or veterinarian who needs an economical general reference, Veterinary Biology and Medicine of Captive Amphibians and Reptiles will more than suffice. For the professional specialist in either field, the book is a necessary complement to a library.

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