EIMERIA WOBESERI SP. N. AND EIMERIA GOELANDISP. N. (PROTOZOA: APICOMPLEXA) IN THE KIDNEYS OF HERRING GULLS (LARUS ARGENTATUS)

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EIMERIA WOBESERI SP. N. AND *EIMERIA GOELANDI* SP. N. (PROTOZOA: APICOMPLEXA) IN THE KIDNEYS OF HERRING GULLS (*LARUS ARGENTATUS*)

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ABSTRACT: *Eimeria wobeseri* sp. n. and *E. goelandi* sp. n. from the kidneys of nestling herring gulls (*Larus argentatus*) are reported. Species descriptions are based on the morphology of sporulated oocysts. Oocysts of one or both species were recovered from 90 of 100 gulls. Nine of 16 gulls rigorously examined were found infected simultaneously with both species. Meronts, gamonts and zygotes were observed histologically in epithelial cells of distal tubules, collecting ducts and ureters. Sporulated oocysts with thin walls and micropylar caps were present within or near collecting ducts and were identified as *E. goelandi* sp. n. An electron-lucent outer layer and electron-dense inner layer of the oocyst wall, the ultrastructure of the oocyst wall at the micropylar cap and endogenous sporulation of *E. goelandi* sp. n. are unique observations for a member of the genus *Eimeria*.

Key words: *Eimeria goelandi*, *Eimeria wobeseri*, kidney, coccidia, herring gull, *Larus argentatus*, new species descriptions.

INTRODUCTION

Renal coccidia have been reported from a wide range of avian host species (Munday et al., 1971; Montgomery et al., 1978; Thompson and Wright, 1978; Obendorf and McColl, 1980; Gajadhar et al., 1983; Leighton and Gajadhar, 1986). To our knowledge, there is only one report of renal coccidia from the Laridae (Creutz and Gottschalk, 1969). In this paper we describe two new species of the genus *Eimeria* in the kidneys of herring gulls (*Larus argentatus*).

MATERIALS AND METHODS

Fifty herring gull nestlings were collected both on 5 and 15 July 1985 on Little Bell Island (Conception Bay, Newfoundland, Canada; 47°35'N, 52°56'W). The birds' mean body weights (one standard deviation) were 343 (43) g and 518 (62) g, respectively, for each collection. The birds were given oral doses of petroleum oils in separate 5-day toxicity experiments (reported elsewhere) and were killed humanely with CO_2 6 to 7 days after capture. Necropsy was performed immediately. The esophagus was cut and the gastrointestinal tracts were reflected without further incision until both kidneys had been removed. The right kidney was placed in 10% neutral-buffered formalin and the left kidney was placed in 2.5% aqueous potassium dichromate. Left kidneys were held at 4 C for 1 to 3 wk. Each was then fragmented in a blender, filtered through cheese-cloth, and the filtrate was held in a petri dish without agitation at 23 to 27 C for 7 to 10 days. Distilled water was added as necessary to maintain fluid volume. The filtrates were then poured into 20-ml containers and transported to the Western College of Veterinary Medicine (University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0). There they were held at 4 C for 1 to 3 days and then were processed by a previously-described technique to isolate oocysts (Gajadhar et al., 1983). Briefly, the filtrates were mixed with a sucrose solution, centrifuged and the top layer of supernatant was removed and examined for the presence of oocysts. Oocysts and sporocysts were measured using a drawing tube and millimeter scale. Measurements were expressed as the mean followed by the standard deviation and range in parentheses.

Fixed tissue was embedded in paraffin and one cross-section approximately 6 μm thick was cut from the cranial, middle and caudal divisions of each kidney selected for histological examination. Sections were stained with hematoxylin and eosin (H&E) and examined. Sections from 20 kidneys were surveyed completely. Sections from a further 34 kidneys were

searched specifically for sporulated oocysts and 33 for stages in the epithelium of the ureter. Sections of distal extrarenal ureter were occasionally present when sections of bursa of Fabricius were studied for another purpose, and these also were examined. Tissues for electron microscopy were selected on H&E sections and embedded in epoxy resin (Jembed 812, J. B. EM Services, Dorval, Quebec, Canada H9R 4S8) directly from the stained 6-μm section (Blank et al., 1970). Ultrathin sections (Ultracut microtome, C. Reichert AG, Vienna, Austria) were stained with uranyl acetate and lead citrate and viewed in a Philips 410LS electron microscope (Philips Electronics, Eindhoven, The Netherlands).

RESULTS

Description of parasites

Two distinctly different types of oocysts were present in the flotation preparations. These are described here as two new species of the genus *Eimeria* (Figs. 1–3).

Eimeria wobeseri sp. n.

(Figs. 1, 3a)

Type host: *Larus argentatus.*

Type locality: Little Bell Island, Newfoundland, Canada (47°35'N, 52°56'W).

Location in host: Kidneys.

Sporulation: The time required for sporulation is unknown. Sporulation of many oocysts was complete when oocysts were first isolated and examined.

Etymology: This species is named in honor of Dr. Gary A. Wobeser, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, to recognize his outstanding and continuing contributions to knowledge of wildlife diseases and the health of wild animals.

Description: Characteristic of the genus *Eimeria*: sporulated oocyst has four sporocysts, each containing two sporozoites (Figs. 1, 3a). Oocysts are elliptical and have a wall which is approximately 1.3 μm thick and consists of two layers. Sporulated oocysts (n = 100) measure 25.1 (2.0, 20.6–29.1) × 19.9 (1.4, 16.2–23.2) μm with an average length : width ratio of 1:1.26. A prominent micropyle is present at the narrow end of the oocyst and two polar bodies, adjacent to each other, are located near the apex. A cluster of several large granules represents the oocyst residuum. Sporocysts (n = 100) measure 9.5 (1.0, 7.3–11.6) × 7.7 (0.9, 5.6–9.8) μm with an average length : width ratio of 1:1.23. They are subspherical to elliptical, have a Stieda body and contain a central sporocyst residuum consisting of several small granules. Curved sporozoites with a central nucleus and a prominent refractile globule are present in each sporocyst.

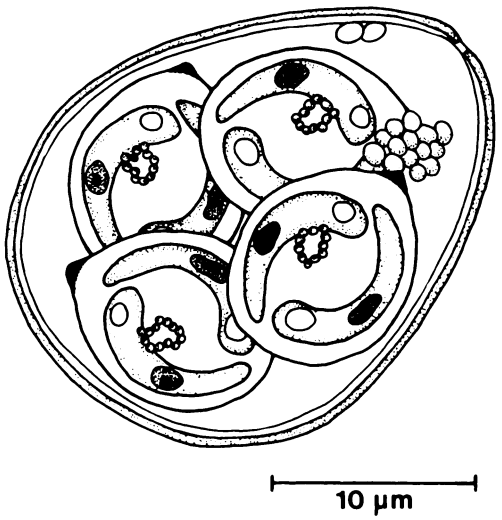

FIGURE 1. Line drawing of a sporulated oocyst of *Eimeria wobeseri* sp. n. from the kidneys of herring gulls.

Eimeria goelandi sp. n.

(Figs. 2, 3b, c)

Type host: *Larus argentatus.*

Type locality: Little Bell Island, Newfoundland, Canada (47°35'N, 52°56'W).

Location in host: Kidneys.

Sporulation: At least some sporulation occurs endogenously within the host's kidney.

Etymology: The specific name is derived from the French word for gull "le goeland."

Description: Characteristic of the genus *Eimeria*: sporulated oocyst has four sporocysts, each containing two sporozoites (Figs. 2, 3b). Oocysts are oval and each is enclosed by a wall which is less than 1 μm thick and is composed of a translucent outer layer and a dense inner layer of equal thickness (Fig. 3c). Sporulated oocysts (n = 25) measure 21.7 (0.72, 20.6–23.5) × 18.1 (1.1, 15.7–19.6) μm and have an average length : width ratio of 1:1.20. A micropyle is present and, in favorable orientations, a micropylar cap is evident. Sporulated oocysts contain a central oocyst residuum composed of a cluster of spherical to subspherical granules. Oval to elliptical sporocysts (n = 25) measure 11.1 (1.0, 8.8–11.8) × 7.1 (0.6, 6.4–8.3) μm with an average length : width ratio of 1:1.56, and have a Stieda body. There is a sporocyst residuum consisting of several granules of various sizes, and curved sporozoites with two refractile globules and a

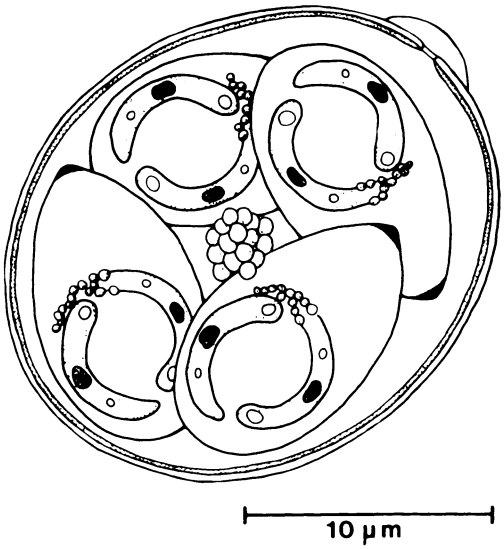

FIGURE 2. Line drawing of a sporulated oocyst of *Eimeria goelandi* sp. n. from the kidneys of herring gulls.

nucleus are located in the posterior two-thirds of the sporocyst.

Differences from other species

Oocysts from *E. wobeseri* sp. n. and *E. goelandi* sp. n. are readily distinguished from those of other avian renal coccidia and from those of the described intestinal *Eimeria* spp. of gulls. Renal oocysts from waterfowl (Anseriformes) are markedly truncated at one end (Gajadhar et al., 1983). Oocysts of *E. gaviae* from the common loon (*Gavia immer*) are large (30.3–40.1 µm) and spherical (Montgomery et al., 1978). Oocysts of *E. fraterculae* from the Atlantic puffin (*Fratercula arctica*) are subspherical and smaller (17.6–20.0 × 17.6–19.6 µm) (Leighton and Gajadhar, 1986). The renal oocysts described as *E. renicola* from the black-headed gull (*Larus ridibundus*) are similar in shape to those of *E. goelandi* sp. n., but they are larger (19.4–34.3 × 18.4–27.8 µm), have thicker walls (1–1.6 µm) and do not have a micropylar cap (Creutz and Gottschalk, 1969). Oocysts of *E. creutzi* and *E. lari* develop in the intestine of gulls and differ in both size and shape from the oocysts of *E. wobeseri* sp. n. and *E. goelandi* sp. n. (Creutz and Gottschalk, 1969; Pellerdy, 1974).

Prevalence

Oocysts were found in the kidneys of 90 of the 100 birds examined. Most contained only small numbers of oocysts; <12 oocysts each were detected in the kidneys of 73 gulls. Over 1,000 oocysts were isolated from

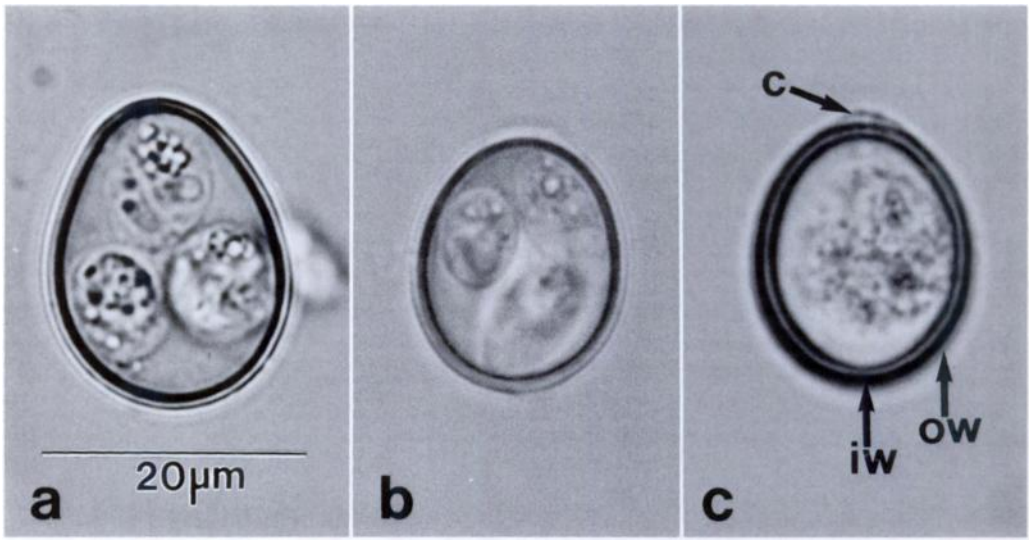

FIGURE 3a–c. Unstained wet preparations of renal oocysts from herring gulls. 3a. Sporulated oocyst of *E. wobeseri* sp. n. 3b. Sporulated oocyst of *E. goelandi* sp. n. 3c. Unsporulated oocyst of *E. goelandi* sp. n. showing the micropylar cap (c), translucent outer layer (ow) and dense inner layer (iw) of the oocyst wall.

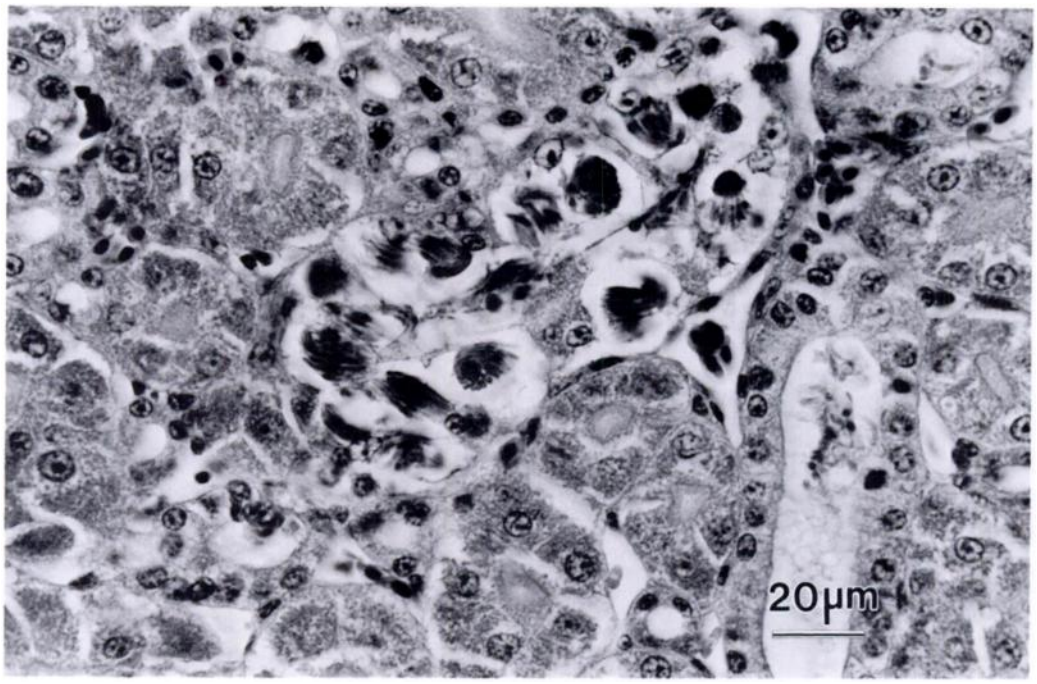

FIGURE 4. Meronts in renal distal tubule cells from a herring gull. H&E.

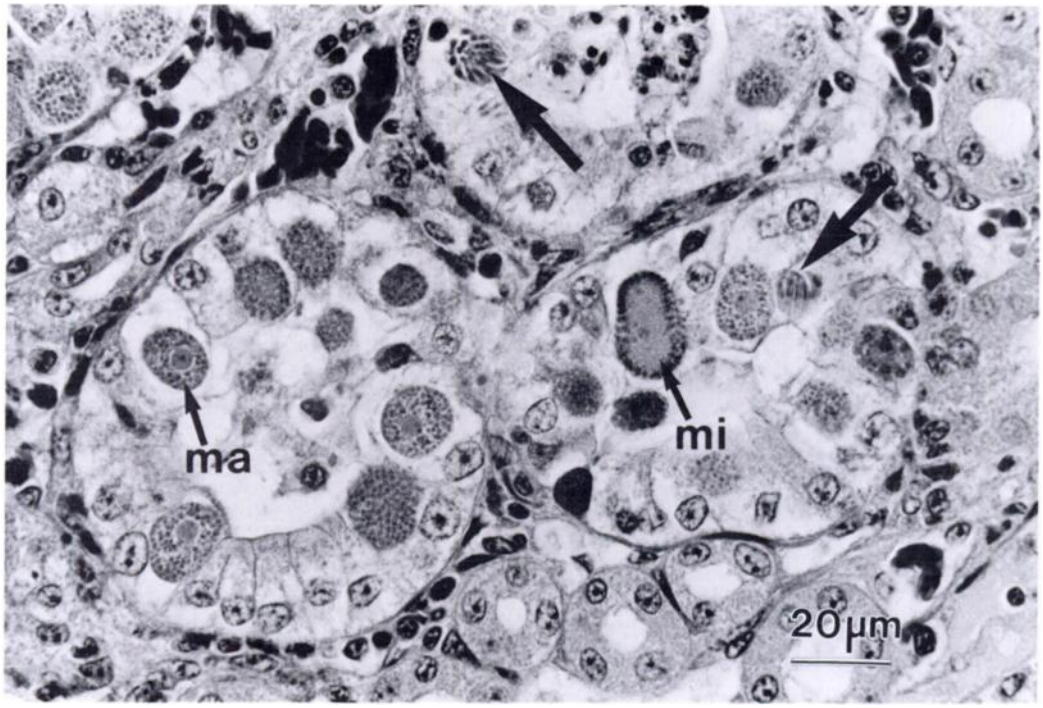

FIGURE 5. Endogenous stages of coccidia in cells of collecting ducts in a renal medullary cone from a herring gull. Meronts (arrows), microgamont (mi) and macrogamont (ma), are evident. H&E.

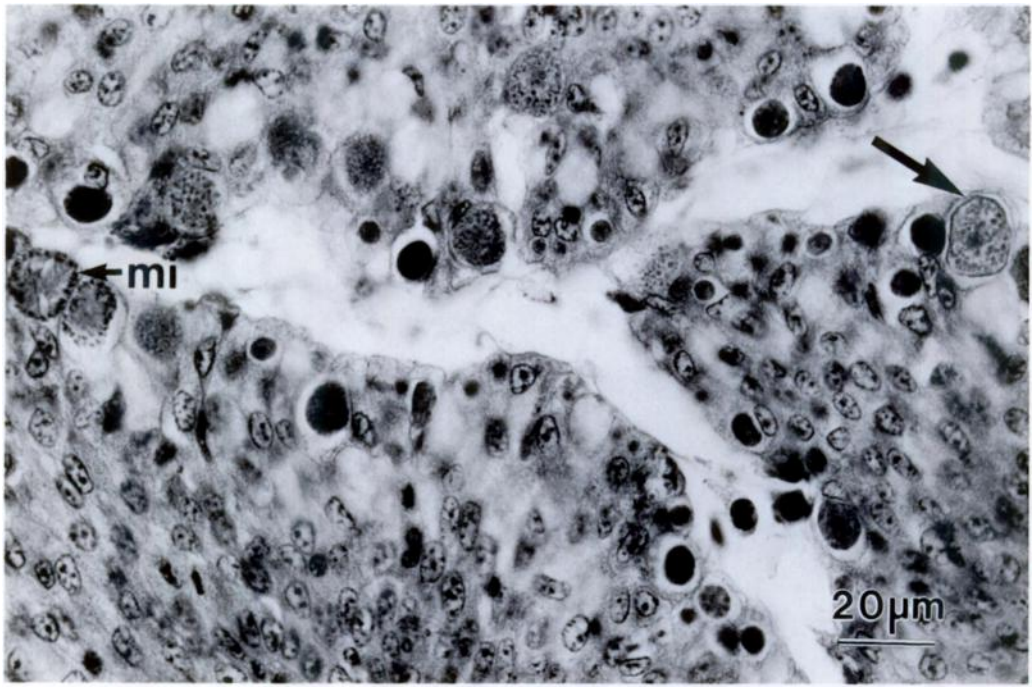

FIGURE 6. Endogenous stages of coccidia in cells of intrarenal ureter from a herring gull. Microgamont (mi) and immature oocyst (arrow) are evident. H&E.

each kidney of 16 birds, and these oocysts were used for species identification. Oocysts of *E. wobeseri* sp. n. were present in 12 and oocysts of *E. goelandi* sp. n. were in 13 of these 16 kidneys. Nine kidneys contained renal oocysts of both species and, in these, the oocysts of *E. wobeseri* sp. n. were isolated in much greater numbers than were those of *E. goelandi* sp. n.

Endogenous stages and pathology

Stages representing merogony, gametogony, oogony and sporogony were evident in histological sections of renal tissues. Each was present at more than one anatomical location. Meronts were regularly present in distal tubule cells (Fig. 4) and clusters of merozoites were seen in the lumen of distal tubules and collecting ducts. Meronts also were seen in cells of collecting ducts in medullary cones (Fig. 5) and in epithelial cells of the ureter. Gamonts were present in the epithelial cells of distal tubules, collecting ducts and in both intrarenal and extrarenal ureters (Figs. 5, 6). A fortuitous section of one kidney contained gamont-bearing epithelium continuously from distal tubule to proximal ureter in one renal lobule. Zygotes undergoing wall formation were present in collecting duct cells and in ureter epithelium (Figs. 5, 6). Oocysts were seen only rarely at these locations. One or more stages of coccidia were present in the epithelium of the ureters of 46 of 53 kidneys examined. Oocysts with internal sporoblasts or sporozoite-containing sporocysts were present in the medullary cones of five of 54 specimens examined (Figs. 7, 8). These oocysts were surrounded by inflammatory cells dominated by multinucleated giant cells, and their precise anatomical locations were seldom identified. Oocysts were often closely adjacent to gamonts and zygotes in collecting duct cells and some were clearly present in the lumina of collecting ducts. The identity of these as sporulated oocysts was confirmed by electron microscopy, which also demonstrated a micropylar cap

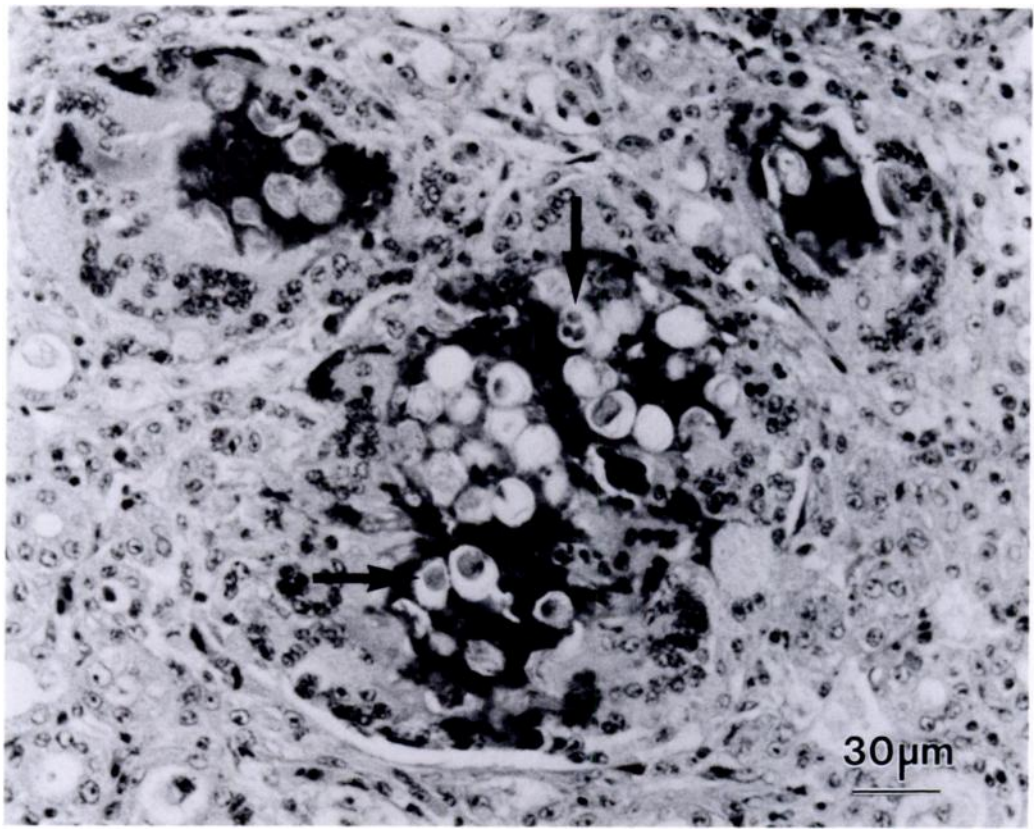

FIGURE 7. Sporulating oocysts (arrows) of *E. goelandi* sp. n. in a renal medullary cone from a herring gull. Clusters of oocysts are surrounded by multinucleated giant cells. H&E.

on the oocyst wall of the sporulated oocysts (Figs. 9, 10).

DISCUSSION

This is the first report of simultaneous infection of a host with two species of renal coccidia. Oocyst morphology allows these two species to be easily differentiated. Oocysts of *E. wobeseri* sp. n. are larger and elliptical whereas those of *E. goelandi* sp. n. are subspherical. Furthermore, oocysts of *E. goelandi* have a micropylar cap, a structure that is only visible when the specimen is precisely oriented.

Developing stages of coccidia were found in the cells of the distal tubules, collecting ducts and ureters. It was not possible to associate each of these stages with one or the other of the two species of mature oocysts isolated by flotation. However, the micropylar cap seen in electron micrographs of the sporulated oocysts present in and around collecting ducts establishes this endogenous stage as *E. goelandi* sp. n. The close anatomical relationship between these sporulating oocysts and meronts, gamonts and zygotes in collecting duct cells suggests that this species may pass through all stages in the collecting duct epithelium. The ureter epithelium was the other anatomical site at which merogony, gametogony and oogony were all observed. The prevalence of endogenous stages at this location in histological sections was 87%. We speculate that the ureter is the major site of gametogony and oogony for *E. wobeseri* sp. n. We cannot infer from our data the probable species to which the other endogenous stages may belong, nor can we determine whether the observed locations of meronts represent all asexual stages of the two species described. Merogony also

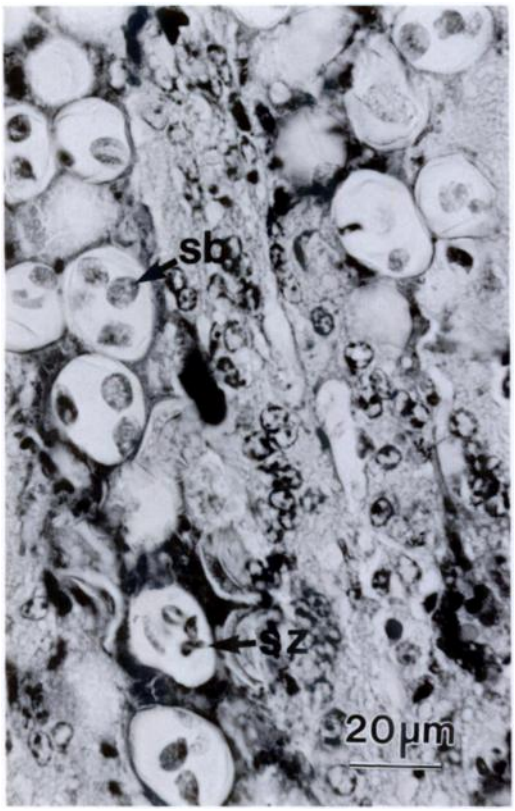

FIGURE 8. Sporulating oocysts of *E. goelandi* sp. n. containing sporoblasts (sb) or sporocysts with sporozoites (sz) in a renal medullary cone from a herring gull. H&E.

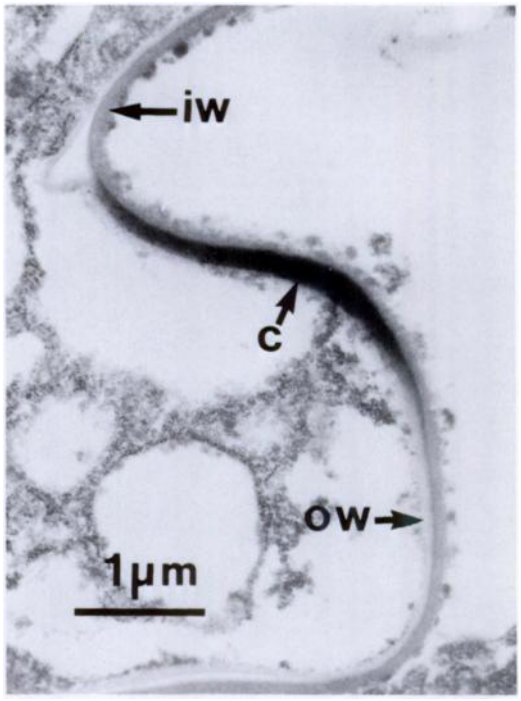

FIGURE 9. Electron micrograph of the collapsed wall of a sporulated oocyst of *E. goelandi* sp. n. in a renal medullary cone of a herring gull. The inner layer (iw) is electron-dense. The outer layer (ow) is electron-lucent. Micropylar cap (c).

may occur in other tissues as appears to be the case in the renal coccidia of little penguins and lesser snow geese (Obendorf and McColl, 1980; Gajadhar and Stockdale, 1986).

Endogenous sporulation in members of the family Eimeriidae is atypical and has not been described previously for species of the genus *Eimeria* (Levine, 1985). Other coccidia undergoing endogenous sporulation and formerly classified in the genus *Eimeria* have been assigned to different genera on the basis of details of morphology and life cycle. For example, a coccidium that sporulates within the liver of killifish and was originally described as *E. funduli* has been assigned to the genus *Calyptospora* (Overstreet et al., 1984). Our present data support assignment of both newly-described species to the genus *Eimeria*. The role of endogenous sporulation in the biology of *E. goelandi* sp. n. remains to be determined. The presence of sporozoites with completely-formed apical complexes within these oocysts suggests that the sporulated oocysts may be infective. Autoinfection without leaving the host is thus a possibility and the unusually thin oocyst wall of this species may facilitate oocyst rupture. Such autoinfection by sporulated, thin-walled oocysts occurs in infections with *Cryptosporidium* (Current and Long, 1983). Shed oocysts are probably immediately infective and this may favor transmission during the short nesting season of the host.

The only endogenous stage of these coccidia that evoked a significant inflammatory response was the sporulating oocysts of *E. goelandi* sp. n. These were associated with a granulomatous response that destroyed surrounding tissue. However, in

each case the amount of tissue affected was small and the effect on the host would have been negligible. Other focal infiltrations with mononuclear inflammatory cells were common in the kidneys of the gulls in this study, but they had no apparent association with any of the stages of the coccidia described.

Eimeria goelandi sp. n. is the first renal coccidium described to have a micropylar cap. In addition, the ultrastructure of the oocyst wall of this species, with an electron-lucent outer layer and an electron-dense inner layer, is unusual. Other eimerians are described as having an electron-dense outer layer and an electron-lucent inner layer in their oocyst walls (Speer et al., 1979). There is some variation in the ultrastructure of oocyst walls among different genera of coccidia. The oocysts of *Isospora canis*, *I. canaria* and *I. serini* have an additional electron-lucent layer outside of the two layers typical of *Eimeria*, and those of *Sarcocystis tenella* consist of a single electron-lucent layer (Speer et al., 1979). The physiological significance of these various oocyst wall configurations remains to be elucidated.

Renal coccidia have not been reported previously in herring gulls. It is possible that *E. wobeseri* sp. n. and *E. goelandi* sp. n. also infect other species of gulls. In experimental studies, renal or enteric coccidia of geese produced patent infections in related species and genera of hosts, and *E. dispersa* of turkeys developed successfully in three different species of Galliformes (Doran, 1978; Gajadhar et al., 1982; Gajadhar et al., 1986). Renal coccidia were reported in black-headed gulls and were named *Eimeria renicola* (Creutz and Gottschalk, 1969). However, sporulated oocysts were not observed and the validity of this generic designation is unconfirmed. Oocysts recovered from kidneys of black-headed gulls differed from those reported here in having no prominent micropyle and no micropylar cap. Oocysts of other species of *Eimeria* reported from herring gulls also differed from *E. wobeseri* sp. n. and *E. goelandi* sp. n. in several details of morphology and were recovered from the alimentary tract (Pellerdy, 1974). Both the host range and the geographic range of the two species of coccidia described in this paper remain to be determined. Endogenous stages similar to those described here were present in a high proportion of herring gull nestlings captured on Great Island and Gull Island, south of St. John's, Newfoundland, Canada in 1982 and 1983; they were absent from four fledgling and 14 adult herring gulls captured in the vicinity of Mount Desert Island, Maine, USA in the summer of 1981 (author's unpubl. data).

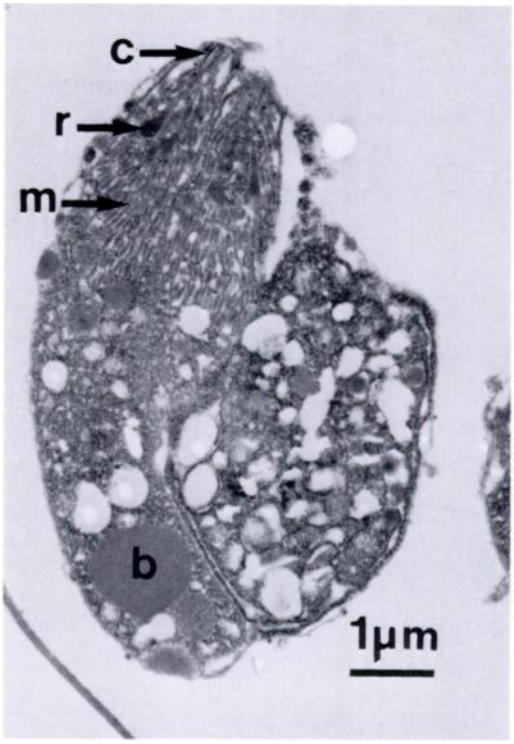

FIGURE 10. Electron micrograph of two mature sporozoites within a sporulated oocyst of *E. goelandi* sp. n. in a renal medullary cone of a herring gull. Conoid (c), rhoptry (r), microneme (m), refractile body (b).

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