Caryospora cheloniae SP. N.: A COCCIDIAL PATHOGEN OF MARICULTURE-REARED GREEN SEA TURTLES (Chelonia mydas mydas)

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Caryospora cheloniae SP. N.: A COCCIDIAL PATHOGEN OF MARICULTURE-REARED GREEN SEA TURTLES (Chelonia mydas mydas)

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Abstract: Caryospora cheloniae sp. n. is described from mariculture-reared green sea turtles (Chelonia m. mydas). The sporulated oocyst has a thin, transparent, single-layered wall which often ruptures, leaving a naked sporulated sporocyst. Oocysts measured 33.8 to 40.1 μm by 11.0 to 14.6 μm (mean 37.4 by 12.8 μm). Greatest concentrations of developmental stages of C. cheloniae were found in the hindgut. Transverse binary fission was observed in dividing tissue stages. Pathologic alterations were most pronounced in the posterior third of the intestines (hindgut). The hindgut lumen was greatly dilated and filled with blood, oocysts and tissue debris. The hindgut wall was thinner than normal and the mucosal folds had sloughed into the intestinal lumen. Free blood escaped from the blood vessels of the tunica propria into the intestinal lumen. Epithelial hyperplasia was pronounced at the margins of denuded mucosal areas. Numerous inflammatory cells infiltrated the infected mucosal surface.

INTRODUCTION

Although a variety of coccidia have been described from reptiles, no species of Caryospora have been described from the Testudinata. The following is a description of a new pathogenic species of Caryospora responsible for the serious economic losses during an epizootic in mariculture-reared young green sea turtles (Chelonia m. mydas) reported previously.

MATERIALS AND METHODS

Material was obtained during an epizootic which occurred during May and June, 1973 in stock hatchlings and juvenile tank-reared green sea turtles (Chelonia m. mydas) maintained in a series of circular fiberglass maintained on Grand Cayman, British West Indies. Affected turtles ranged in age from approximately 4 to 8 weeks. Tissue sections of the internal organs, the intestinal tract, and intestinal contents were collected during necropsy from dead, moribund and clinically normal turtles during the course of the outbreak.

All tissues were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin. In addition, intestinal sections were stained by the

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Oxidative Schiff-Aniline Blue-Orange G method for coccidia. Freshly-harvested feces and necrotic intestinal mucosa containing unsporulated oocysts were placed in 250 ml Erlenmeyer flasks, containing 125 ml of 2.5% potassium dichromate in seawater at 25°C and placed on a Burell wrist action shaker for agitation. This suspension was examined daily at irregular intervals over a period of 98 h. At each examination the number of sporulated and unsporulated oocysts and free sporocysts present in the above suspension were counted and the percentage sporulation for that interval was calculated and recorded. Suspensions of oocysts and sporocysts to be measured, photographed and utilized for morphologic study, were centrifuged and then washed three times in a sterile solution of synthetic sea salts. Measurements were made with a 95X apochromatic objective and a filar micrometer 10X eye piece. Photographs were taken by direct, phase contrast and interference-contrast illumination. Unstained glutaraldehyde fixed oocysts were examined by an electron microscope.

RESULTS

Oocysts of a new species of Caryospora were recovered from the pure infection of the green turtles. A description of the observed developmental stages of the organism and histopathologic alterations of infected hosts tissues are given below.

Oocyst

Unsporulated oocysts were elongate, ellipsoidal in shape, and with smooth walls. The sporont filled the inner space as a fine light green granular material. As sporulation progressed, granular material of the sporont became more compact and condensed in the central portion of the oocyst to form a sporoblast (Fig. 1).

Sporulated oocysts were monosporocystic and octozoic; however, during the process of sporulation, the fragile double layered oocyst wall usually disintegrated, releasing the sporulated sporocyst. Only 6/1000 sporulated sporocysts examined were contained within oocysts.

Five oocysts measured 33.8 to 40.1 μm by 11.0 to 14.6 μm (mean 37.4 by 12.8 μm). The wall thickness of these oocysts measured 0.3 to 0.6 μm (mean 0.5 μm). The surface of the five oocysts walls were irregular, asymmetrical and lacked uniformity when compared to each other.

FIGURE 1. Partially sporulated oocyst of Caryospora cheloniae, showing central condensations of the granular contents during formation of a sporoblast. (2000X).
FIGURE 2. Monosporocystic, octozoic sporulated oocyst of *Caryospora cheloniae* showing thin transparent single layered oocyst wall. (2000X).

FIGURE 3. A single sporulated sporocyst of *Caryospora cheloniae* showing central residuum, sporozoites with refractile bodies and pointed apical ends, domed stieda body, substiedal lenticular body, and fissure in base of wall with attached external adhering debris. (2000X).

(Fig. 2). Oocyst residuum, polar body and micropyle were absent. The long axis of both oocyst and sporocysts often were curved to varying degrees, like a sausage or cucumber. Such curvature altered length-width index measurements. Of 100 sporocysts measured individually, the length varied from 26.2 to 44.1 μm, the width from 10.6 to 17.3 μm (mean 34.5 × 12.7); length-width ratio was 1.5 to 4.2 (mean 2.8). Twenty-five single-layered sporocysts walls measured 0.5 to 0.9 μm (mean 0.7); the walls appeared yellow or colorless with a fine black internal border. The stieda body of the sporocyst was distinctive and consisted of a thin layer of the sporocyst wall that formed an elevated dome over a colorless lenticular body (substiedal body) at the base of the dome (Fig. 3). Twenty-four of the domes measured 0.7 to 3.5 μm (mean 2.0 μm) in height and 2.0 to 5.7 μm (mean 4.6 μm) in width. Twelve lenticular bodies measured 0.95 to 1.6 μm (mean 1.3μm) in height and 2.2 to 5.0 μm (mean 3.8 μm) in width. The end of the sporocyst opposite the stieda body featured a fine median fissure that traversed the sporocyst wall perpendicularly from its internal to external surface.
FIGURE 4. Section of infected hindgut epithelium showing cords of dividing macrogamonts. (1500 X).

FIGURE 5. Section of infected hindgut epithelium showing early trophozoite stage (G), immature macrogamonts (I) along basement membrane, dividing macrogamonts (D), mature macrogamonts (M), microgametocyte (MI), oocyst (O) and proliferating epithelial cells (P). (1000 X).

FIGURE 6. Excysted sporozoite showing flexed attenuated apical end, prominent elongated raised posterior refractile body with ring-like structure at posterior pole. (5000 X).

The internal and external ends of the fissure were elevated as tiny knob-like projections (Figs. 3 and 7). On electron microscopic examination of the above knob-like structures, the external structure appeared as a pointed outward projection of the sporocyst wall and the internal structure as an elevated inner collection of fine granular material. It was not clear whether the additional external adhering debris, seen on light microscopy, was herniated sporocyst contents or vestiges of the oocyst wall that remained attached (Figs. 3 and 7).

Twenty-five of the sporocyst residua measured 8.7 to 17.1 by 6.9 to 12.1 μm (mean 11.4 × 9.7 μm) and consisted of spherical bodies composed of large coarse granules at the circumference. The center consisted of less dense finer granules. The residuum tended to be
central in location with the sporozoites extending towards the poles of the sporocyst from each side of the residuum. Upon storage, the residuum tended to migrate or become displaced towards one pole and the sporozoites displaced towards the other end. Also, the large granules composing the circumference of the sporocyst residuum often became detached and floated freely in the sporocyst cavity.

Twenty-five sporozoites measured within the sporocysts were 12.6 to 16.6 by 2.0 to 3.7 \( \mu \text{m} \) (mean 13.3 by 2.8 \( \mu \text{m} \)). Two excysted, completely extended, sporozoites measured 15.6 to 16.0 \( \mu \text{m} \) by 3.8 \( \mu \text{m} \) (mean 15.8 by 3.8 \( \mu \text{m} \)). Sporozoites were club or cigar-shaped with a blunt, rounded end and a short apical pointed end that was capable of elongation and flexion. Twenty-five posterior refractile bodies, 2.2 to 3.8 \( \mu \text{m} \) by 1.1 to 3.3 \( \mu \text{m} \) (mean 2.9 by 2.5 \( \mu \text{m} \)), were prominent elongated elevated structures. Frequently a ring-like structure, behind the posterior refractile body, was observed. Twenty-five of the anterior refractile bodies had diameters from 1.1 to 2.0 \( \mu \text{m} \) (mean 1.6 \( \mu \text{m} \)) (Fig. 6). A small, poorly defined round nucleus was noted between the refractile bodies.

Complete sporulation was observed to occur in 19.5 hrs. at 25 C. Oocysts continued to sporulate after this time and the greatest percentage increase was noted between 23.5 and 73.5 hrs. After this time, little change was noted (Table 1).

Developmental Stages

The earliest and smallest stage noted in the infected epithelium was a short cigar-shaped meront with one rounded and one pointed end. The rounded end contained the nucleus with a prominent nucleolus. Ten such meronts measured 9.2 to 10.7 by 7.9 to 8.6 \( \mu \text{m} \) (mean 10.3 by 8.4 \( \mu \text{m} \)). The nuclei were 2.1 to 3.6 \( \mu \text{m} \) (mean 2.9 \( \mu \text{m} \)) in diameter.

The above meronts could be found in loosely arranged groups of 2 to 6 organisms in the middle of the epithelial layer. Binary fission frequently was observed during this stage together with transverse division of the nucleus, nucleolus and cytoplasm, followed by elongation and separation of the dividing parts (Figs. 4 and 5). Division was completed by separation of the cell walls perpendicular to the long axis of the dividing segments. However, the segments remained attached, end-to-end, following division, forming cords of divided cells. One end of the cord was closer to the intestinal lumen. Segments of the cord closer to the basement membrane were larger, well-differentiated,
and could be recognized as immature or mature macrogamonts or microgamonts. The more distal segments, those closer to the intestinal lumen, were smaller, less differentiated and more actively dividing.

Immature macrogamonts had thin walls, a fine, eosiophilic granular cytoplasmic reticulum and a small, round, centrally placed eosinophilic nucleus containing a prominent basophilic nucleolus. Mature macrogamonts could be differentiated from the above by their larger size, elliptical shape, lighter-colored and less dense cytoplasm, smaller nucleolus, and the formation of a vacuole around the macrogamont serving to isolate it from the surrounding tissue. Mature macrogametes were closer to the lumen surface.

Ten macrogametes measured 28.6 to 37.2 µm by 10.0 to 13.6 µm (mean 32.2 by 12.2 µm). The nuclei ranged from 3.6 to 5.7 µm (mean 4.5 µm) in diameter; with nucleoli measuring 1.4 to 2.2 µm (mean 1.8 µm) in diameter (Fig. 5).

Immature microgamonts were very dark colored, oval to round shaped inclusions within the cytoplasm of epithelial cells. As the microgamonts increased in size, the nucleus of the parasitized host epithelial cell was eccentrically displaced, became elongated and was compressed by the growing parasite.

Developing microgametocytes enlarged into cystic structures containing dark staining, basophilic granules at the periphery and a central dark-colored spherical body. Growth continued until the microgametocyte ruptured at the lumen surface, releasing myriads of biflagellated curved rod-like microgametes, less than 0.25 µm in length. Ten mature microgametocytes measured 30.7 to 42.9 by 16.4 to 27.2 µm (mean 37.6 by 23.9 µm) (Fig. 5).

Following fertilization, the unsporulated oocyst was surrounded by a large clear space formed during migration from the ruptured host cell into the intestinal lumen. The fertilized oocyst was transformed into a basophilic structure with coarse, dark, cytoplasmic granules and a dark nucleus without a visible nucleolus (Fig. 5).

### Pathology

Pathologic alterations were most pronounced in the posterior third of the intestines (hindgut), which included the posterior portion of the small intestines and the large intestines. The greatest concentration of infective stages of coccidia were observed in the hindgut. The hindgut lumen was greatly dilated and filled with blood, oocysts and tissue debris. The hindgut wall was thinner than normal. The wall was thinnest...
where the epithelial folds had sloughed into the lumen. The tips of the folds of the hindgut were also denuded of their epithelial cover and free blood was escaping from the blood vessels of the tunica propria into the intestinal lumen. Remnants of the epithelium were markedly altered and epithelial hyperplasia was pronounced at the margins of the denuded areas. Collections of densely packed, poorly defined, irregularly-shaped epithelial cells with large nuclei and scant cytoplasm were present in these areas. Numerous inflammatory cells, including eosinophils, were present in the inflamed mucosal layer.

The anterior third (foregut) and the middle third (midgut) of the intestinal tract evidenced little structural change, although individual developmental stages of the coccidial organism could be seen within individual epithelial cells at the tips and sides of the villi of the small intestines.

DISCUSSION

C. cheloniae is an economically important pathogen of mariculture-reared green sea turtles (C.m.mydas). The importance of this disease for the free-ranging endangered species has yet to be evaluated. In addition to being the first Caryospora reported from turtles, the life cycle is distinctive because of its division by transverse binary fission and the structure of the sporulated oocyst.

C. cheloniae produces marked tissue destruction in the hindgut and is the first serious coccidial pathogen reported from turtles. More experimental study is needed to further define the life cycle, pathogenesis, treatment and control.

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LITERATURE CITED


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