Surface Ultrastructure of *Eimeria tenella*, *E. dispersa*, and *E. meleagrimitis* Motile Forms

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ABSTRACT: Three previously undescribed surface structures were detected when *Eimeria tenella*, *E. dispersa*, and *E. meleagrimitis* sporozoites were examined by high-resolution scanning electron microscopy. There were apical projections that varied in number from two to five, depending on the species examined. These projections may connect with the rosette-rhoptry complex and aid in host-cell penetration. Secondly, there were papillalike structures arranged in longitudinal rows extending from the apex of the sporozoite to its midpoint; on merozoites of *E. tenella* the papillae were found the entire length of the organism. Additionally, longitudinal ridges are described and are suggested to be surface manifestations of the internal microtubules.

Sporozoites, the infective motile forms of bovine coccidia (*Eimeria*), flex and glide when penetrating host cells (Fayer and Hammond, 1967). This type of movement suggests a complex ultrastructure, various aspects of which have been reported by numerous workers and reviewed by Scholtyseck (1979). D’Hase et al. (1977) described subpellicular microtubules that extended from the anterior polar ring to the midpoint of *Eimeria* sporozoites. Although Porchet-Hennere’ and Ponchel (1974) and Mehlhorn and Heydorn (1978) reported superficial longitudinal striations or riblike structures on the pellicular surface of *Sarcocystis tenella* merozoites, such structures have not been reported on the sporozoites of poultry coccidia.

The penetration of host cells is essential to the survival of the parasite, yet very little is known about the actual mechanisms of penetration. Jensen and Edgar (1976) suggested that the saclike rhoptries in bovine coccidia probably functioned to secrete a substance that aided in penetration. Dubremetz and Torpier (1978) observed an “apical rosette” lying anterior to the rhoptries but below the surface of the sporozoite pellicle. With scanning electron microscopy (SEM), Vetterling et al. (1971) demonstrated that the conoidal complex of *Eimeria tenella*, a poultry coccidium, protruded in some sporozoites.

The present study was designed to examine sporozoites of poultry coccidia and use high resolution SEM to locate surface structures that may aid movement and penetration.

Materials and Methods

Oocysts of *Eimeria dispersa*, *E. tenella*, and *E. meleagrimitis* were collected from experimentally infected birds and sporulated and excysted as described by Doran and Vetterling (1967). Excysted sporozoites were purified by the glass-bead column method of Doran (1970). Sporozoites were fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.3, for 3 hr, washed 3× in buffer (0.05 M cacodylate with 0.2 M sucrose, pH 7.3) for 15 min, and postfixed for 1 hr (1% OsO₄ in 0.05 M cacodylate with 0.15 M sucrose). The temperature of all solutions throughout the processing was 4°C. The fixed sporozoites were washed 3× in distilled H₂O, suspended in 25% ethanol, and placed in a 10-ml syringe with an
attached 3-μm-pore polycarbonate membrane. A graded series of ethanol (25, 50, 75, 95, 100%) was passed over the retained sporozoites and was followed by four passages of 100% ethanol at room temperature to complete the dehydration. The membrane with the attached sporozoites was dried in a critical-point drying apparatus (Madden and Tromba, 1976) and a piece was mounted on a specimen stub with cellulose adhesive. The specimens were coated with 20 nm of 60/40 Pd-Au alloy in an ion sputtering device.

Merozoites of *E. tenella* only, were prepared by removing cecal tissue from infected birds and examining the cut surface as described by Madden and Vetterling (1977).

The specimens were examined at 100 kV accelerating voltage in a JEOL 100-C¹ transmission electron microscope with scanning coils.

**Observations and Discussion**

When examined by high-resolution SEM, sporozoites of *E. dispersa*, *E. meleagrititis*, and *E. tenella*, had several common morphological structures on the pellicular surface. These structures, however, projections and papillae, and longitudinal ridges, have not been previously reported. The projections were on the apex of most of the sporozoites examined. On the apex of *E. dispersa* there was an evenly spaced, circular arrangement of five projections. These could be observed on apices of both protruded (Fig. 1) and nonprotruded (Fig. 2) conoids. Apical projections on sporozoites with protruded conoids were 60 nm long and jutted out prominently from the apex. Sporozoites with nonprotruded conoids were 18 nm long and appeared blisterlike. The whole sporozoites of *E. meleagrititis* were blunter than those of *E. dispersa* and had three apical projections that were not as distinct as those of *E. dispersa* (Fig. 3). The sporozoites of *E. tenella* were the bluntest of the three species examined, and the number of projections varied from two to three (Fig. 4).

Dubremetz and Torpier (1978) using freeze-etch techniques reported apical rosettes in the Pe fracture face (Branton et al., 1975) of *Eimeria nieschulzi* sporozoites, which lies below the pellicular surface of the plasmalemma and above a small vesicle surrounded by an irregular circle of openings that might be rhoptry ducts. These ducts are believed to secrete substances that aid the sporozoite in the penetration of host cells (Jensen and Edgar, 1976).

The presence of the apical projections immediately above the reported location of the apical rosettes suggest that these projections may be extensions of the rhoptry-rosette complex and as such may be involved in penetration. These projections may serve as surface conduits or ducts for the hypothesized secretory product of the rhoptry. The projections themselves could also be the secretory product adhering to the surface of the sporozoite. Because the projections were not observed on all sporozoites, it is possible that their presence is transitional, or that the projections may have been removed during preparation of the material for SEM. Raised areas or small papillae were also observed on the pellicular

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¹ Mention of a trade name, proprietary product, or vendor does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable.
surface of sporozoites and merozoites (Figs. 1, 2, 5). These were arranged in parallel rows running longitudinally from the anterior to the midpoint of the sporozoite. On merozoites, the papillae appeared more uniformly spaced and extended nearly the entire length of the merozoite, giving it the appearance of a cucumber (Fig. 5). The presence of the normally formed erythrocyte observed with this specimen suggests that these papillae are not artifactual (Fig. 5). These papillae have not been previously described for the genus *Eimeria* but they may be a common feature for the apicomplexa, since they are discernible in micrographs of *S. tenella* (Porchet-Hennere' and Ponchel, 1974).

Longitudinal ridges extending from the polar ring to a point about midway on the pellicle were observed regularly on sporozoites (Figs. 6, 7). These ridges appear to be analogous to those found on merozoites of *S. tenella* and called superficial striations (Porchet-Hennere’ and Ponchel, 1974) or riblike structures (Mehlhorn and Heydorn, 1978). D’Hase et al. (1977) have shown that the subpellicular microtubules in *Eimeria* sporozoites extend from the polar ring to midpoint. The longitudinal ridges may be surface manifestations of the internal microtubular organization.

The posterior half of the sporozoites examined, except for the caudal end, appeared smooth and was swollen, probably because of support from the underlying refractile granule (Figs. 6–8). However, some sporozoites swollen from an anterior refractile granule still had the rough surface (Fig. 8). Figures 6 and 7 are lower-magnification micrographs of the sporozoites in Figures 1 and 2 and show that the morphology of sporozoites is uniform.

The posterior end of the sporozoites in all three species was blunt compared with the anterior end. As an example, in *E. dispersa* sporozoites (Figs. 6–8) the posterior end, measuring 1 μm in diameter just distal to the posterior refractile granule, was twice as blunt as the anterior end, which measured 0.5 μm at the polar ring and 0.35 μm at the protruded conoid.

We have elucidated the surface morphology of some motile forms of *Eimeria*, and have attempted to correlate the anterior projections with the rhoptries and apical rosettes which are believed to aid in host cell penetration. Further investigation is needed to determine whether the number of apical projections varies between other species of coccidia. Additionally, serial sectioning and transmission electron microscopy might enable us to determine further the true nature of the projections.

**Literature Cited**


